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Increased Expression of Proinflammatory Cytokines in Chronic Lesions of Human Cutaneous Leishmaniasis

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The nature of the host cellular immune response largely determines the expression of disease following infection with the intracellular protozoans *Leishmania* spp. In experimental animals control and resolution of infection are mediated by gamma interferon and tumor necrosis factor alpha (TNF-α), whereas disease progression is associated with the production of interleukin 4 (IL-4), IL-5, IL-10, and transforming growth factor beta (TGF-β). We have analyzed the profile of cytokine gene expression directly in the lesions of 13 patients with localized cutaneous leishmaniasis due to *Leishmania mexicana*. All but one patient had a single lesion, and the time of evolution ranged from 8 days to 18 months. Cytokine gene expression was quantitated by reverse transcriptase PCR and interpolation from a standard curve. Gamma interferon, TNF-α, IL-1α, IL-6, IL-10, and TGF-β gene expression was present in all samples. IL-3 and IL-4 gene expression was barely detectable in 1 and 3 of 13 samples, respectively. IL-2 and IL-5 mRNAs were not found. A significant increase in the expression of IL-1α, TNF-α, IL-10, and TGF-β was observed in late lesions (≥4 months) compared with that in early lesions (≤2 months). Because of their inhibitory effects on macrophage function, the expression of IL-10 and TGF-β may play a role in the immunopathogenesis of chronic cutaneous leishmaniasis.

Infection with the intracellular protozoans *Leishmania* spp. causes a spectrum of clinical manifestations depending on the parasite species and the host immune response. Localized cutaneous leishmaniasis (LCL) caused by *Leishmania mexicana* is common in Mexico and parts of Central America. Infection with *L. mexicana* may be subclinical or manifest as cutaneous lesions which may persist for months to years or heal spontaneously within weeks to a few months (1a). In general, lesions persisting longer than a few months are unlikely to heal spontaneously (1).

Resistance to *Leishmania* infection is mediated by cellular immune mechanisms (26). Resolution of murine *Leishmania major* infection is associated with the capacity of T cells (T_H1 subset) to generate gamma interferon (IFN-γ) (8, 17, 37, 40) and tumor necrosis factor alpha (TNF-α) (22, 42). Exacerbation and progression of disease are associated with the activation of T_H2 cells and production of interleukin 4 (IL-4) (17, 18, 40), IL-5 (40), and IL-10 (18). Production of transforming growth factor beta (TGF-β), probably by infected macrophages, has also been associated with an increase in lesion size in the murine model (4).

In humans the healing of lesions in LCL appears to be associated with resistance to experimental or natural reinfection (reviewed in reference 23); however, the mechanisms of resistance have not been clearly defined. Mitogen- or antigen-induced lymphokines (most importantly IFN-γ) from peripheral blood mononuclear cells from patients with LCL or mucosal leishmaniasis have been shown to activate human monocytes to kill intracellular *Leishmania* parasites (6, 31, 33). Peripheral blood mononuclear cell responses are directed against a large number of diverse *Leishmania* antigens which vary in their ability to induce IFN-γ production (25).

The cellular immune response within the cutaneous lesion is of primary importance in the outcome of infection. Immunohistochemical studies have demonstrated that active LCL lesions are infiltrated with large numbers of $CD4^+$ and $CD8^+$ T lymphocytes (32) and T lymphocytes bearing the γδ receptor (28). A very small percentage of intralesional T cells have been shown to produce IL-2 and IFN-γ both in situ (29, 32, 34) and in response to *Leishmania* antigens in vitro (34). Analysis of intralesional cytokine gene expression in *Leishmania braziliensis* infection showed that the T_H1 cytokine mRNAs (IL-2, IFN-γ, and lymphotoxin) were present in cutaneous lesions but IL-4, and to a lesser extent IL-5 and IL-10, mRNAs were abundant in mucosal lesions (35). In another study of LCL, in which the *Leishmania* species was not specified, prominent IFN-γ but weak or absent IL-2, IL-4, IL-5, and IL-10 expression was observed (5). In the present study, the intralesional expression of cytokine genes in patients with LCL caused by *L. mexicana* was quantified by reverse transcriptase PCR. Expression of IL-2, IL-3, IL-4, and IL-5 was minimal or absent, whereas IL-1α, IL-6, IL-10, TGF-β, IFN-γ, and TNF-α mRNAs were abundant. The expression of TNF-α, IL-1, IL-10, and TGF-β was significantly increased in chronic lesions compared with expression in early lesions. These results indicate that the differential and temporal expression of cytokines in situ may have a role in the pathogenesis of this disease and that the pattern of cytokine expression may be unique to the particular *Leishmania* species.

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MATERIALS AND METHODS

Patients. Thirteen patients from an area of the Yucatan, Mexico, where LCL caused by *L. mexicana* is endemic were studied. These patients were identified and treated as part of a multidisciplinary research project centered at the Universidad Autonoma de Yucatan in Merida, Mexico. The patients were diagnosed by a combination of clinical, epidemiological, serological, histopathological, and parasitological criteria (15). Where possible, parasitological confirmation of the diagnosis was obtained by culture of the tissue biopsy samples or aspirate in biphasic medium consisting of an agar slant containing 30% rabbit blood overlaid with Locke's solution. The ages of the patients ranged from 10 to 29 years. All but one of the patients were male. Twelve patients had a single lesion, and one patient had two lesions. The time of evolution of the lesions ranged from 8 days to 18 months (median, 4 months). The skin biopsy samples (taken at the indurated edge of the cutaneous lesion) were part of diagnostic studies and thus were obtained prior to the institution of therapy.

Histopathology. Punch biopsy samples were formalin fixed, and tissue sections were examined following routine hematoxylin and eosin staining. The cellular infiltrate was characterized according to the presence or absence of amastigotes, polymorphonuclear leukocytes, disorganized granulomas, and giant cells. Semiquantitative assessment of the numbers of lymphocytes and plasma cells (each graded as making up less than, equal to, or greater than 50% of the cellular infiltrate) and macrophages (graded as 0, 1+, or 2+) within the lesion was also carried out. In two subjects (502 and 505) the quantity of tissue was not sufficient to carry out histopathological analysis.

Isolation of RNA. Tissue from lesion biopsies was immediately frozen in liquid nitrogen, where it was stored until analysis. Each frozen tissue specimen was pulverized with a stainless steel cylinder-piston apparatus under liquid nitrogen, and the powder was transferred to RNA lysis buffer (TriSolve; BIOTECX, Houston, Tex.) containing guanidinium isothiocyanate. Total RNA was isolated according to the manufacturer's instructions. The cylinder and piston were thoroughly washed and then soaked in 10% sodium hypochlorite for 5 min after each sample homogenization to prevent carryover of any nucleic acid from one sample to another (36).

Reverse transcription and PCR. Quantitative reverse transcriptase PCR was carried out as previously described (24). First-strand DNA was synthesized at 37°C for 1 h with 18 μl of RNA in RNase-free dH_2O, 8.0 μl of 5× reverse transcription buffer (250 mM Tris-HCl [pH 8.3], 375 mM KCl, 50 mM dithiothreitol, 15 mM $MgCl_2$), 2.0 μl (20 U) of RNAsin (Promega, Madison, Wis.), 4.0 μl deoxynucleoside triphosphate (dNTP) mix (10 mM [each] dATP, dCTP, dGTP, and dTTP), 4.0 μl (0.5 μg) of random hexadeoxynucleotide primers, and 4.0 μl (0.8 U) of Moloney murine leukemia virus reverse transcriptase (GIBCO BRL, Gaithersburg, Md.). One to two microliters of first-strand cDNA or purified cloned template cDNA at various concentrations was added to a PCR mix containing 5 μl of 10× reaction buffer (500 mM KCl, 100 mM Tris-HCl [pH 8.3], 1.5 mM $MgCl_2$), 4 μl of dNTP mix (1.0 μM [each] dATP, dCTP, dGTP, and dTTP), 0.25 μl (1.25 U) of *Thermus aquaticus* (*Taq*) DNA polymerase (Boehringer Mannheim), and sterile dH_2O to a total volume of 49.5 μl per reaction. Each primer (2.5 μl) was added to give a final primer concentration of 100 pmol/ml. The mixture was subjected to DNA amplification with a Gene Machine II thermal cycler (USA/Scientific Plastics, Ocala, Fla.) set at 95°C for 1 min, 50°C for 2 min, and 72°C for 2 min for a total of 25 cycles. [^{32}P]dATP (3,000 Ci/mmol; DuPont/NEN, Boston, Mass.) (5 μCi) was added to each reaction after 20 cycles. Care was taken in the preparation of the PCRs to prevent any nucleic acid contamination, and a negative control was included in each assay. The PCR product mixture (8 μl) was separated on a 0.75-mm-thick urea-acrylamide gel, and the gel was fixed in 40% methanol–10% acetic acid–50% dH_2O for 30 min and dried under vacuum at 60°C for 1 h. The dried gels were then placed in a BetaScope counter (Betagen, Waltham, Mass.), and the appropriate PCR product bands were counted for 5 min. Standard curves were generated by fivefold dilution of the cytokine cDNA templates prior to amplification. The input cytokine cDNA from the biopsies was quantitated by interpolation from the exponential linear portion of the standard curve as previously described (24). Because of the minute quantity of RNA isolated from a single biopsy sample, most cytokines were analyzed only once.

Oligonucleotides and DNA templates. Oligonucleotide primers were synthesized on a DNA synthesizer from Applied Biosystems, Inc. (Foster City, Calif.), and purified by reverse-phase high-pressure liquid chromatography. Primers were designed by us (24) from published sequences with 5′ primers (plus strand) and 3′ primers (minus strand) chosen from within coding regions such that they were approximately 20 bp in length, had similar melting temperatures, and flanked a single intron so that any amplification of contaminating genomic DNA would be readily identified. Oligonucleotide probes specific for exon and intron sequences were synthesized in a similar fashion for use in Southern blot analysis. Exon probes were chosen from sequences to be amplified by the PCR primer pair, and intron probes were chosen from sequences within the intron flanked by the PCR primer pair. Nucleotide sequences of sense and antisense PCR primer pairs not previously described are as follows: IL-1α, GTTCCTCCATT GATCATCTG and GGCTTAAACTCAACCGTCTC; IL-6, TGTGAAAGCAGCAAAGAGGC and TTCTGCAGGAAC TGGATCAG; TGF-β, GGAAATTGAGGGCTTTCGCCT and GAAGCAATAGTTGGTGTCCAG; CD3δ, ATAGCAC GTTTCTCTCTGGC and ATGTCTGAGAGCAGTGTTCC; and NKG2-c, AGAAGTGAGTCTGGCCCAGG and GATT TCTAGGACCTCGGCAG.

The cloned cDNA templates for IL-2, IL-3, IL-4, IL-5, IL-10, TNF-α, IFN-γ, and β-actin were cut from plasmids and purified as previously described (23). The IL-1α, IL-6, TGF-β, CD3δ, and NKG2-c cDNA templates were prepared as follows: pBR327 containing the human TGF-β insert (ATCC 59954) was digested with *Eco*RI to yield a 2,138-bp template; pMG-5 containing the human IL-1α insert (ATCC 65258) was digested with *Bam*HI to yield a 2,400-bp template; pT7.7 containing the human IL-6 insert (ATCC 68636) was digested with *Bgl*II and *Bam*HI to yield a 1,450-bp template; pcD-1 containing the human CD3δ insert (ATCC 57084) was digested with *Xba*I and *Pst*I to yield a 700-bp template; pAprM8 containing the NKG2-c insert (kindly provided by J. P. Houchins, University of Minnesota) was digested with *Eco*RI to yield a 1,200-bp template.

DNA blot analysis. Southern blotting was performed after agarose electrophoresis of the IL-2 PCR products followed by denaturation and neutralization of the gels. The DNA was then transferred to a nylon membrane (Nytran; Schleicher & Schuell, Keene, N.H.), UV cross-linked (Stratalinker; Stratagene, La Jolla, Calif.), and hybridized with intron- and exon-specific oligonucleotide probes. The hybridization signal was visualized with a chemiluminescence detection system (ECL; Amersham, Arlington Heights, Ill.) according to the manufacturer's instructions.

Statistical analysis. Differences in the levels of gene expres-

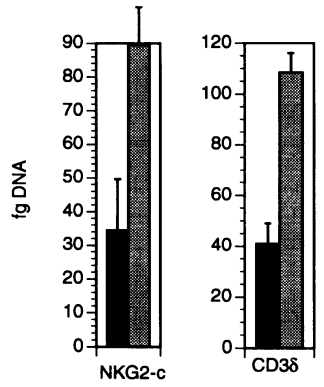

FIG. 1. Comparison of NK-cell and T-cell gene expression in early (≤2-month duration) (■) and late (≥4-month duration) (▨) LCL lesions. Values are expressed as the mean and standard error of the quantitated PCR products normalized to β-actin PCR product.

sion between the early and late lesions were analyzed by using both an unequal-variance *t* test and Wilcoxon rank sums. Differences were considered significant if a *P* of <0.05 was achieved.

RESULTS

Histopathology. *Leishmania* amastigotes were identified in histological sections in 8 of 11 biopsy samples. The intralesional cellular infiltrate was made up predominantly of lymphocytes, macrophages, and plasma cells. Semiquantitative analysis indicated that plasma cells and macrophages were present in similar numbers in all of the biopsy samples. Lymphocytes made up greater than 50% of the cellular infiltrate in three of four of the early lesions (<2-month duration) but only one of seven of the late lesions (>4-month duration). In contrast, polymorphonuclear leukocytes were identified in one of four early lesions but five of seven late lesions. Disorganized granulomas were present in both early (two of four) and late (six of seven) lesions.

Analysis of intralesional gene expression. Total RNA was isolated from snap-frozen skin biopsy samples and reverse transcribed to cDNA. Quantitation of β-actin expression demonstrated only slight (twofold) variation in the total RNA content of the biopsy samples. Total T-cell mRNA was quantified by analysis of CD3δ expression. Natural killer (NK) cell mRNA was quantified by amplification of the 5′ portion of the NKG2-c gene which is expressed in NK cells and rarely in T cells (19). There were striking differences, however, among the different patient biopsy samples in the amount of total T-cell- and NK-cell-specific mRNA (37- and 20-fold differences, respectively). A significant increase was observed in the T-cell- and NK-cell-specific mRNA in late lesions (>4-month duration) compared with that in early lesions (<2-month duration) ($P = 0.008$ and $P = 0.02$, respectively) (Fig. 1). Because the NKG2-c gene may be rarely expressed in T cells, these results should be interpreted cautiously.

The in situ cytokine gene expression was normalized on the basis of the quantitation of either the β-actin (monokine) or CD3δ (lymphokine) PCR product. The expression of IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IFN-γ, TNF-α, and TGF-β in the lesions of the 13 patients is detailed in Table 1. IL-2 and IL-5 transcripts were not detected in the lesions of any patient. To ensure that the inability to detect IL-2 did not result from technical error, aliquots of several of the biopsy samples were

TABLE 1. Quantitation of intralesional cytokine gene expression by reverse transcriptase PCR

Patient	Lesion duration (mo)[a]	Amt (fg) of cDNA[b] for:							
		IL-1	IL-3	IL-4	IL-6	IL-10	IFN-γ	TNF-α	TGF-β
473	1	7.6	0	0.008	18.8	1.2	7.4	0.5	56
482	1	0.4	0	0	27	2.6	9.2	0.3	48
487	1	4.5	0	0	69	2.1	7.7	0.3	69
488	2	0.5	0	0	0.8	0.1	33	0.2	16
502	0.3	2.4	0.3	0	4.6	0.2	0.7	0.3	70
505	0.3	3.8	0	0.03	2.8	0.6	0.2	1.1	123
483	6	0.4	0	0	6	0.6	14	0.2	23
486	4	2.2	0	0	3.8	0.8	4.1	0.3	101
490	9	20	0	0.1	21.6	11	12.2	3.2	>400
491	4	8.7	0	0	75	6	12.5	1.8	256
492	5	14	0	0	32	4.5	8.9	1.9	173
493	4	0.8	0	0	12	1.1	3.4	1.2	88
499	18	27.4	0	0	96	8.2	17.6	6.8	342

[a] Prior to biopsy.
[b] Values are from interpolation from a standard curve and have been normalized to expression of CD3δ (IL-3, IL-4, and IFN-γ) or β-actin (all other cytokines). IL-2 and IL-5 cDNAs were not found in the lesions of any patients.

spiked with purified IL-2 cDNA and amplified. There was no inhibition of the amplification of the spiked template when amplified in the presence of the biopsy sample-derived nucleic acid. Additionally, the IL-2 PCR products were analyzed by Southern blotting using intron- and exon-specific oligonucleotide probes. A single band was barely detected by both probes (data not shown), indicating that a minute amount of genomic DNA but no mRNA was present. The mRNAs of IL-3 and IL-4 were weakly expressed in 1 and 3 of 13 samples, respectively.

Expression of IFN-γ, TNF-α, IL-1α, IL-6, IL-10, and TGF-β genes was identified in all the biopsy samples. There was significantly greater expression of TNF-α ($P = 0.045$ and $P = 0.048$ by unequal-variance *t* test and Wilcoxon test, respectively), IL-10 ($P = 0.03$ and $P = 0.058$), and TGF-β ($P = 0.02$ and $P = 0.03$) transcripts in late lesions compared with that in early lesions (Fig. 2). There was a trend toward greater expression of IL-1α and IL-6 in the late lesions, but this was not statistically

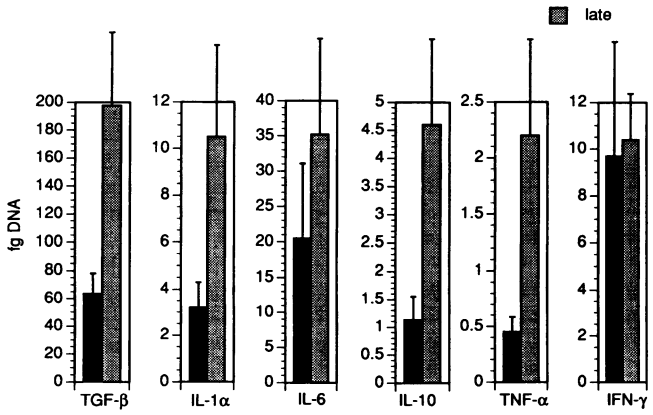

FIG. 2. Comparison of cytokine gene expression in early (≤2-month duration) (■) and late (≥4-month duration) (▨) LCL lesions. Values are expressed as the mean and standard error of the quantitated PCR products normalized to the β-actin or CD3δ PCR product as described in Table 1.

TABLE 2. Correlation matrix of intralesional cytokine gene expression

Cytokine	Correlation coefficient of:				
	TGF-β	TNF-α	IL-1α	IL-6	IL-10
TNF-α	0.84				
IL-1α	0.89	0.93			
IL-6	0.55	0.66	0.63		
IL-10	0.95	0.79	0.87	0.59	
IFN-γ	0.07	0.21	0.18	0.17	0.19

significant. There was strong correlation between the expression of IL-1α, TNF-α, IL-10, and TGF-β (Table 2). IL-6 expression did not correlate as strongly with that of the above cytokines. IFN-γ mRNA (normalized to the CD3δ PCR product) was strongly expressed in both groups of patients (Fig. 2), and there was no correlation between IFN-γ expression and that of the other cytokines. There was considerably more IFN-γ mRNA in late lesions than in early lesions when it was normalized to total mRNA (β-actin), most likely because of the increased numbers of T cells (as determined by CD3δ expression) in the late lesions.

DISCUSSION

Analysis of cytokine gene expression within the lesions of LCL due to *L. mexicana* revealed a predominance of mRNA for IFN-γ and the proinflammatory cytokines IL-1α, IL-6, IL-10, TNF-α, and TGF-β. In spite of the prominence of T lymphocytes in the cellular infiltrate (29), the interleukin mRNAs typically associated with T-cell activation (IL-2, IL-3, IL-4, and IL-5) were largely absent. The lack of detection of these cytokines is not likely to be due to insensitivity of our methodology, as we have previously shown that expression can be readily detected in a very small number of activated T cells (24). Rather, it seems that the intralesional T cells were silent in the expression of a number of cytokine genes. This suggests that either a subset of T cells lacking the capacity to produce these cytokines is represented within the lesion or there is considerable down-regulation of cytokine synthesis within the cellular infiltrate. Other investigators have also noted a paucity of T-cell cytokines within the lesions of cutaneous leishmaniasis and leprosy. An in situ study of cutaneous lesions due to *L. braziliensis* demonstrated that while T cells made up 75% of the total cells in the lesions, only 5% expressed the IL-2 receptor and only 0.3% of all cells expressed IFN-γ mRNA (34). Similarly, a T-cell infiltrate is prominent in the lesions of tuberculoid leprosy but less than 0.5% of cells stained positively for IL-2 (27). In a recent analysis of cytokine gene expression in LCL and mucosal lesions caused by *L. braziliensis*, T_H1 cytokine mRNAs (IL-2, IFN-γ, and lymphotoxin) were observed in LCL lesions (IL-2 was weakly expressed). IL-4 and, to a lesser extent, IL-5 and IL-10 mRNAs were found in mucosal lesions (35). In a similar study in which the infecting parasite species was not specified, these investigators found prominent IFN-γ and TNF-β expression in LCL lesions but IL-2, IL-4, IL-5, and IL-10 expression was minimal or absent (5). In contrast to those in our study, all of these patients had lesions lasting less than 5 months. The absence or minimal expression of IL-2, IL-4, and IL-5 mRNAs and prominence of IL-10 mRNA in our study of lesions due to *L. mexicana* may reflect differences in the pathogenesis and/or patient populations studied.

Our results are also distinct from the cytokine profiles observed in the draining lymph nodes of murine cutaneous leishmaniasis. In the murine model of *L. major* infection, the inability to control and heal infection is associated with the expansion of the T_H2 subset and expression of IL-4, IL-5, and IL-10 (17, 18, 40). Spontaneous healing in resistant or immunized mouse strains is associated with the expansion of the T_H1 subset with expression of IL-2 and IFN-γ (17, 18, 37, 40). We were unable to detect expression of IL-4 or IL-5 in even the most chronic (but still localized) lesions. Transient expression early in the course of lesion development, which could have an immunoregulatory effect, cannot be excluded. A recent study of patients with active visceral leishmaniasis likewise failed to detect IL-4 and IL-5 expression in bone marrow cells (20). The absence of IL-4 and IL-5 mRNAs in these lesions may reflect a difference in the immunopathogenesis of human and murine disease or differences in the inflammatory makeup of the skin compared to lymph node. The skin, in particular, the epidermal keratinocytes, is a rich source of proinflammatory cytokines which contribute to the pathogenesis of a number of inflammatory skin diseases (38).

A number of cell types present within the lesion, including lymphocytes, macrophages, and epidermal cells, have the potential to produce these cytokines. There was no obvious correlation between the numbers of intralesional macrophages or lymphocytes and the pattern of cytokine expression. The absence of coexpression of IL-2 and IFN-γ mRNA suggests that the IFN-γ mRNA is not derived from the classically defined T_H0 or T_H1 $CD4^+$ T-cell subsets. Activated NK cells could be the source of the IFN-γ mRNA (43). The expression of IFN-γ mRNA correlated only weakly with the levels of expression of either T-cell (CD3δ)- and NK-cell (NKG2-c)-specific genes.

The proinflammatory cytokines (IL-1α, IL-6, IL-10, TNF-α, and TGF-β) are primarily products of mononuclear phagocytes and fibroblasts, but most may also be produced by B and T lymphocytes (2). Macrophages have been shown to produce IL-1α (7), TNF-α (42), and TGF-β (4) in response to *L. major* infection. In the skin, epidermal keratinocytes are a prominent source of IL-1, IL-6, IL-10, TNF-α, and TGF-β (13, 21), and IL-1 and IL-6 are also synthesized by Langerhans cells (39). Immunohistochemistry or in situ hybridization studies are needed to identify the source of these cytokines.

The increased expression of the macrophage-inhibitory cytokines IL-10 and TGF-β in late lesions compared with that in early lesions suggests a role for these cytokines in the immunopathogenesis of chronic disease. This increased expression may be due to an increased level of cellular activation or a relative increase in the number of cytokine-producing cells within the lesion infiltrate. Because tissue for cytokine analysis was obtained from a single site in each subject, we cannot exclude the possibility of some variation due to a sampling artifact. The subjects were treated with chemotherapy after the tissue was obtained for these studies, so there is no way of knowing which patients might have subsequently healed spontaneously.

Both IL-10 and TGF-β have been shown to down-regulate macrophage function. The intralesional expression of these cytokines could therefore promote the persistence of the intracellular pathogen. IL-10, which may be produced by T cells or monocytes/macrophages, inhibits cytokine synthesis by T_H1 cells and cytotoxic T-lymphocytes but not T_H2 cells in mice (14). In humans, however, cytokine synthesis by both T_H1 and T_H2 cells is inhibited (9). This inhibition is mediated indirectly through interference with antigen presentation by monocytes, by either down-regulation of class II major histocompatibility complex expression (10, 11) or decreased pro-

duction of costimulatory signals (12). The production of IL-10 during active visceral leishmaniasis (16, 20) indeed has been demonstrated to contribute to the suppression of *Leishmania*-induced lymphoproliferative responses (16). The absence of the IL-2, IL-3, IL-4, and IL-5 mRNAs in the lesions we studied thus may be due to the prominent expression of IL-10. IFN-γ synthesis by $CD4^+$ and $CD8^+$ T cells is also potently inhibited by IL-10 (9, 30); however, human T_H1 T-cell clones have recently been shown to produce both IFN-γ and IL-10 (9). Thus, the IFN-γ and IL-10 transcripts detected in our study may be derived from the same T cells.

TGF-β has been shown to decrease IFN-γ-induced class II major histocompatibility complex expression on monocytes (8) and blocks macrophage activation by IFN-γ (44). Production of TGF-β by *Leishmania*-infected macrophages results in increased susceptibility to and progression of murine leishmaniasis (4). It has also been shown to diminish the capacity of macrophages to inhibit the intracellular growth of *Trypanosoma cruzi*, another intracellular protozoan (41). Because the synthesis of TGF-β is posttranscriptionally regulated (3), measurement of gene expression may not correlate with biologic activity. Our results indicate that at least the population of cells having the capacity to synthesize TGF-β is expanded in the late lesions.

Our results suggest that the intralesional expression of the inhibitory cytokines IL-10 and TGF-β may contribute to the chronicity of infection. Intralesional IL-4 and IL-5 expression was not observed and apparently does not contribute to persistence of the established lesion. IFN-γ expression, which was prominent in the lesions studied, is not sufficient in itself to effect healing. Cytokine expression in the very early stages of infection may differ from this pattern observed in the established cutaneous lesion. Longitudinal studies which identify changes in cytokine expression associated with clinical outcome are needed to confirm these results.

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