Differential Expression of Chemokines in Patients with Localized and Diffuse Cutaneous American Leishmaniasis

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The abundance of macrophages in localized cutaneous leishmaniasis (LCL) and diffuse cutaneous leishmaniasis (DCL) lesions and differences in the composition of T cell subsets indicate involvement of cell-specific chemotaxis processes. The expression of macrophage chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α and -1β, RANTES (regulated on activation, normal T cell expressed and secreted), I-309, and interleukin-8 were investigated in lesions of patients with LCL or DCL. In LCL, high levels of MCP-1 and moderate levels of MIP-1α were detected. In DCL, MCP-1 expression was significantly lower and MIP-1α expression was predominant. All other chemokines investigated were minimally expressed or absent. These findings suggest that MCP-1 and MIP-1α are responsible for the recruitment of macrophages and T cells in cutaneous leishmaniasis. The results show that self-healing LCL is associated with higher levels of MCP-1, which may stimulate macrophage microbicidal mechanisms, and nonhealing DCL is associated with higher levels of MIP-1α.

Cutaneous leishmaniasis is initiated by the bite of an infected sandfly and deposition of protozoan Leishmania parasites in the skin. In the mammalian host, the organisms are obligate intracellular and invade macrophages and Langerhans cells for sequestration and replication [1, 2]. The site of infection in the dermis is characterized by a massive lymphohistiocytic infiltrate. Macrophages play a central role in the course of infection because they serve not only as host cells for Leishmania species but also as antigen-presenting cells that modulate the specific cellular immune response and, after appropriate activation, as effector cells for intracellular killing of the parasites.

While the cellular composition of the lesional infiltrate in cutaneous leishmaniasis has been characterized in detail [3–7], the signals initiating and regulating the local accumulation of discrete cell subsets are only poorly understood. Chemokines of the C-C family (β chemokines) are attractive candidates for this function because they selectively attract monocytes and certain T cell subsets. The β chemokine family includes monocyte chemoattractant protein (MCP)-1 [8], also known as monocyte chemotactic and activating factor (MCAF) [9] or human JE protein [10], macrophage inflammatory protein (MIP)-1α [11] and -1β [12], RANTES (regulated on activation, normal T cell expressed and secreted) [13, 14], and I-309 [15]. The expression of individual β chemokines has been demonstrated in chronic inflammatory processes, such as psoriasis [16], delayed-type hypersensitivity reactions related to sarcoidosis and tuberculosis [17], gingival inflammation [18], and schistosome granuloma formation [19]. Interleukin (IL)-8, a potent neutrophil chemoattractant, has also been implicated in T cell chemotaxis [20, 21]. However, it is not known whether particular inflammatory conditions are associated with a characteristic array of locally produced chemokines and whether their pattern correlates with the severity of disease.

To elaborate the potential involvement of chemotactic factors in the pathology of cutaneous leishmaniasis, we analyzed their expression in localized cutaneous leishmaniasis (LCL), the most benign form of the disease spectrum with self-healing skin ulcers, and in diffuse cutaneous leishmaniasis (DCL), which is characterized by extensive unremittent spreading of nodular lesions. Both forms of infection are caused by the same Leishmania species and are thought to reflect the patient’s immune response to the parasite. We used in situ hybridization to identify and localize the mRNA expression of the chemokines MCP-1, MIP-1α and -1β, RANTES, I-309, and IL-8 and did immunohistology on serial sections to detect the presence of the corresponding protein and determine its correlation with the recruitment of specific leukocyte subsets.
Materials and Methods

Patients. Eleven patients with LCL and 4 with DCL were studied. All lived in Mexico in areas in which leishmaniasis is endemic (Campeche, Tabasco, and Veracruz). Only _Leishmania mexicana mexicana_ has been isolated in these areas [22]. LCL lesions had evolved for 2–12 months. The average duration of DCL was 5 years. The diagnosis was established by clinical, histologic, microbiologic (cultivation and histologic examination), immunologic (Montenegro skin test), and molecular (polymerase chain reaction [PCR]) parameters. Montenegro skin tests were done by injecting 0.1 mL of leishmanin intradermally and measuring the skin reaction after 48 h. A swelling >4 mm in diameter was considered a positive response. All LCL patients studied had positive Montenegro skin tests, whereas DCL patients were completely unresponsive to the leishmanin injection. DCL patients had multiple nodular lesions randomly distributed over the integument; however, of the 11 LCL patients, 8 had 1, 2 had 2, and 1 had 3 lesions. For 7 of 11 LCL lesions that were superficially ulcerated, additional infection with fungal or bacterial pathogens was excluded. To prove _Leishmania_ infection, _L. m. mexicana_–specific PCR was done on all biopsies as described [23]. LCL biopsy samples were collected before initiation of therapy. DCL patients had received their last 30-day course of pentavalent antimony therapy 3–4 months before biopsy collection but were experiencing relapses.

Biopsy specimens. Scalpel skin biopsy specimens were collected under local anesthesia. Biopsies from healthy volunteers and from patients with psoriasis with pronounced infiltration of macrophages and T lymphocytes and well-characterized chemokine expression were used as controls. One-third of each tissue sample was fixed in 4% paraformaldehyde and processed according to routine histologic procedures. LCL lesions showed a strong infiltrate of macrophages and lymphoid cells. The presence of granulomatous infiltrates with lack of caseation necrosis was variable but generally low. In DCL sections, there was a uniform infiltration pattern with paratized macrophages and lymphoid cells. In contrast to LCL lesions, the epidermis was flattened and showed a complete loss of the rete ridges. The remaining two-thirds of the tissue samples were snap-frozen in optimal cutting temperature (OCT) compound (Miles, Naperville, IL), and 4-μm cryostat sections were used for immunohistology and in situ hybridization. For studies of the phenotype of inflammatory cells and the chemokine profile, only lesions with intact epidermal structures and, most importantly, without superinfection were used.

Antibodies. For immunohistologic analyses, the following monoclonal antibodies (Mabs) were used at the indicated dilutions: anti-CD1a (1:1000; Coulter, Krefeld, Germany) recognizing Langerhans cells; anti-CD3 (1:100; Becton Dickinson, Sunnyvale, CA) reacting with the T cell receptor–associated CD3 antigen; anti-CD4 (1:100; Dako, Hamburg, Germany) recognizing helper T cells; anti-CD8 (1:100, Dako) recognizing cytotoxic T cells; anti-CD22 (1:200, Dako) recognizing B cells; anti-CD45 (1:100, Becton Dickinson) directed against the lymphocyte common antigen on all human leukocytes; anti-CD68 (1:2000, Dako), which recognizes monocytes and macrophages; anti–neutrophil elastase (1:200, Dako); anti–MIP-1α (1:10; Promega, Madison, WI). Polyclonal antibodies directed against MCP-1 were purchased from Genzyme (Cambridge, MA). Polyclonal antibodies to _L. mexicana_ were raised in rabbits by subcutaneous and intramuscular injections of promastigotes in complete Freund’s adjuvant, followed by several boosters of intact promastigotes in PBS and collection of the serum. Biotin-conjugated sheep anti-mouse immunoglobulin at 1:200 (Amersham, Braunschweig, Germany) and biotin-conjugated donkey anti-rabbit immunoglobulin at 1:1000 (Dianova, Hamburg, Germany) were used as second-stage reagents.

Immunohistology. For immunocytochemical labeling of tissue sections, a three-step streptavidin-peroxidase procedure was used [24]. The cryostat sections were thawed onto gelatin-coated slides, air-dried, and fixed in acetone (4°C, 10 min). Because of their fragility, DCL sections were postfixed in 4% paraformaldehyde in PBS (4°C, 10 min). OCT compound was washed from slides with Blotto (a solution containing 5% skim milk powder and 0.1% Tween 20 in PBS, pH 7.4), and nonspecific binding sites were blocked with 20% heat-inactivated fetal calf serum (GIBCO Laboratories, Eggenstein, Germany) for 30 min at room temperature (RT). Thereafter, the sections were incubated with the appropriate first-step antibody in Blotto overnight (4°C), followed by incubation with the respective biotin-conjugated second-step antibody (1 h, RT) and preformed streptavidin-biotin peroxidase complex (Dako) for 1 h (RT). Sections were thoroughly rinsed between each incubation step. Finally, labeling was visualized with the substrate 3-amin-9-ethyl-carbazole (AEC; Sigma, Deisenhofen, Germany). The AEC solution contained 0.2 mg/mL AEC previously dissolved in _N,N_-dimethylformamide (final concentration, 5%) and 0.005% _H_2O2 in acetate buffer (50 mM, pH 5). For control purposes, the first-step antibodies were omitted or replaced by an irrelevant isotype-matched immunoglobulin. These stainings consistently yielded negative results.

In DCL biopsies, only fixation with 4% paraformaldehyde in PBS (20 min, RT) but not with acetone allowed satisfactory histologic preservation of the fragile sections. Therefore, immunohistologic labeling of these sections was only possible with leukocyte-specific antibodies after microwave treatment but not with chemokine-specific antibodies. This additional procedure did not alter the sensitivity since LCL lesions subjected to the same treatment showed an identical quantitative composition of all lesional leukocyte subsets tested in this study.

Preparation of 35S-labeled RNA probes. Full-length cDNA probes were provided by T. Yoshimura (National Cancer Institute, Frederick, MD; MCP-1), Genetics Institute (Cambridge, MA; MIP-1α), M. Krangel (Duke University Medical Center, Durham, NC; IL-1, IL-6), V. Mielke (Universität Ulm, Germany), and C. Weissmann (Universität Zürich, Switzerland; IL-8). Subcloning of specific DNA fragments in plasmids with SP6/T7 (Promega) or T3/T7 initiation sites (BlueScript SK/KS; Stratagene, La Jolla, CA) was done by standard protocols [25]. In vitro transcription of sense and antisense probes was done as previously described [16]. Briefly, after linearization of plasmid DNA with appropriate restriction enzymes, 35S-labeled sense and antisense probes were obtained by in vitro transcription using T3, T7, and SP6 RNA polymerases (Boehringer, Mannheim, Germany) together with ATP, GTP, CTP (Boehringer), and 35S-UTP (Amersham) as substrates. The original linearized template cDNA was eliminated by DNase treatment, and protein components were eliminated by sequential phenol extraction steps. Thereafter, alkaline hydrolysis of the 35S-labeled RNA probes was done for 30–50 min at 60°C.
in a carbonate buffer (pH 10.2) according to the formula: time (minutes) = \((L_0 - L_f)/0.11 \times L_0 \times L_f\), where \(L_0\) = initial length in kilobases (kb), and \(L_f\) = final size in kb [26]. The resulting size reduction of the probes (average length, 50–150 bp) facilitated their penetration into the cells. The size was checked by electrophoresis on a denaturing 6% polyacrylamide gel. Finally, after several ethanol precipitation steps, the radioactive riboprobes were adjusted to a specific activity of \(2 \times 10^6\) cpm/\(\mu\)L in 0.1 M TRIS- HCl, pH 7.5, containing 1 mM EDTA. Generally, 70%–95% of UTP radionucleotides were incorporated into the RNA probe.

Hybridization procedure. In situ hybridization was done as described by Müller et al. [27]. Paraformaldehyde-fixed cryostat sections (4% paraformaldehyde in PBS, 20 min) were treated with proteinase K (1 \(\mu\)g/mL; Boehringer) for 30 min at 37°C to facilitate the binding of radioactive probes to cellular target mRNA, refixed in paraformaldehyde, acetylated with acetic anhydride in 0.1 M triethanolamine (pH 8.0, 10 min), dehydrated in graded concentrations of ethanol, and air-dried. Thereafter, the sections were overlaid with 20 \(\mu\)L of hybridization solution (50% formamide, 300 mM NaCl, 20 mM TRIS-HCl, pH 8.0, 5 mM EDTA, 1 X Denhardt’s solution, 10% dextran sulfate, 100 mM dithiothreitol, and \(2 \times 10^5\) cpm/\(\mu\)L heat-denatured radioactive probe). The slides were mounted with coverslips, sealed, and hybridized at 46–47°C for 12–16 h. Every antisense and sense (control) probe was hybridized with at least three sections from the same biopsy. RNase treatment was used as an additional control and consistently abrogated specific hybridization signals. After the hybridization, non-hybridized probes were removed by several high-stringency washing procedures with 50% formamide solution containing 2 X saline-sodium-citrate buffer (Sigma) and 5 mM EDTA at 54–57°C with constant stirring (30 min). For further minimization of nonspecific background, noncomplementary unhybridized single-stranded probe RNA was digested with RNase A (20 \(\mu\)L/mL) and RNase T1 (1 \(\mu\)g/mL; Boehringer) for 30 min at 37°C. For autoradiography, slides were dipped in Kodak NTB-2 solution (1:2 in 800 mM ammonium acetate) and exposed for 1–5 weeks at 4°C. For evaluation and localization of the leukocyte subsets, we made immunohistologic stainings using a panel of MAb s that recognize macrophages and T, B, and Langerhans cells and neutrophils. In LCL lesions, most of the leukocytes were gathered in the upper part of the dermis, where they formed dense clusters. In these clusters, most cells consisted of macrophages (70%–90%) and CD3 cells (21%–8%) with a predominance of CD4 (not CD8) cells. All other leukocyte subtypes (neutrophils and B and Langerhans cells) were singly scattered and contributed only marginally to the massive leukocyte accumulation in LCL lesions. The relative percentages of all leukocyte subtypes investigated are summarized in figure 2A. Compared with LCL, the distribution and composition of leukocytes were different in DCL lesions. The leukocytes were evenly distributed in the whole dermal compartment and were mainly macrophages (70% ± 10%). There were significantly fewer T lymphocytes in DCL lesions (12% ± 6%, \(P < .08\); figure 2A). The reduced proportion of T cells in DCL could be attributed to a decreased number of CD4 cells (\(P < .06\)) that led to an inverse ratio of CD4:CD8 cells compared with LCL lesions. In contrast, the difference in the percentage of CD8 cells in LCL and DCL was not significant (\(P < .6\); figure 2A).

MCP-1 expression in Leishmania lesions. The study of the composition of infiltrating leukocytes in LCL and DCL lesions revealed that mononuclear cells, particularly macrophages and T lymphocytes, are the predominating cell populations. Assuming that specific chemotactic processes may be responsible for the recruitment of these leukocyte subsets, we investigated the presence of chemokines that attract monocytes and macrophages, T lymphocytes, or both. With in situ hybridization and MCP-1 antisense probes, we detected strong cell-associated signals in the dermal and epidermal compartments of LCL lesions (figure 3A, 4A). In the dermis, high levels of MCP-1...
mRNA were mainly encountered in the areas of leukocyte clustering that were frequently seen in the upper dermal compartment. In the epidermis, MCP-1 mRNA expression was restricted to the basal layer above the dermal infiltrate (figure 4A). To demonstrate that the high levels of MCP-1 mRNA are actually transformed into protein, we did immunohistologic labeling. With this process, we detected strong immunoreactivity with MCP-1–specific antibodies, but not with irrelevant antibodies, in areas where high levels of MCP-1 mRNA were expressed in successive sections (figure 3A, B).

Immunohistologic labeling with macrophage-specific (anti-CD68) and T cell–specific (anti-CD3) antibodies demonstrated that the foci of high levels of MCP-1 transcripts and immunoreactivity were associated with a preponderance of infiltrating monocytic and lymphocytic cells (figure 3). This supports the notion that MCP-1 mRNA is transcribed into functional protein and attracts monocytes, macrophages, and lymphocytes in vivo. In control sections of normal skin or of infected skin treated with sense probes, neither hybridization signals nor detectable. As a positive control for evaluation of the signal density, we used psoriatic lesions with pronounced infiltration of macrophages and lymphocytes and strong MCP-1 expression in the basal keratinocytes of the rete ridges [16]. Comparison of dermal MCP-1 mRNA expression in psoriasis and LCL tissues showed that MCP-1 levels are substantially higher in LCL lesions: 5.9% of all dermal cells expressing MCP-1 compared with <2% in psoriasis.

In contrast to LCL lesions, the number of dermal cells expressing MCP-1 mRNA was low in DCL lesions (2.2% ± 0.3%). Moreover, expression of MCP-1 in the epidermis (flattened with loss of the rete ridges) was not detectable in any of the lesions investigated, and clustering of MCP-1 mRNA–positive cells was less obvious (figure 4C). Figure 2B shows the percentage of MCP-1–expressing dermal cells in LCL and DCL. The data demonstrate that the level of MCP-1 expression in LCL is about three times higher than in DCL, and the differences are highly significant (5.9% ± 1.3% vs. 2.2% ± 0.3%, P < .01).

**MIP-1α expression in Leishmania lesions.** Expression of MIP-1α mRNA was detected in LCL and DCL lesions, and cell-associated hybridization signals were exclusively in the dermis. In LCL lesions, MIP-1α expression was moderate and mainly focal (figures 4B, 5A). As for MCP-1, the areas of MIP-1α mRNA expression corresponded with the regions of
MIP-1α immunoreactivity (figure 5B) and with the localization of CD68 macrophages (figure 5C). Moreover, in situ hybridization with MCP-1 and MIP-1α antisense probes on serial sections revealed that mRNA expression of both chemokines was clustered in the same inflammatory foci (figure 4A, B).

Of particular interest, MIP-1α expression in DCL lesions was much higher than in LCL, and mRNA-expressing cells were evenly distributed in the whole dermal compartment (figure 4D). Thus, the most striking finding of this study was that the levels of MIP-1α expression in LCL and DCL lesions (2.7% ± 0.5% vs. 4.4% ± 1.3%, P < .02) were reciprocal to those of MCP-1 as revealed by in situ hybridization of serial sections (figure 4). Figure 2B quantifies MCP-1 and MIP-1α mRNA-expressing cells in LCL and DCL lesions, respectively. In LCL lesions, the expression of MIP-1α was significantly lower than MCP-1, whereas in DCL lesions, MIP-1α expression was much higher than MCP-1.

Expression of MIP-1β, RANTES, I-309, and IL-8 in Leishmaniasis lesions. In addition to MCP-1 and MIP-1α, several related chemokines exhibit monocyte and lymphocyte chemoattractant properties (or both) that could influence the composition of inflammatory cells in leishmanial lesions. Therefore, we hybridized the biopsy sections with antisense probes of MIP-1β, RANTES, I-309, and IL-8. In contrast to the high abundance of cells expressing MCP-1 and MIP-1α mRNA, however, these chemokines were expressed only minimally by a few scattered cells in the dermis of LCL and DCL lesions (figure 2B). Only MIP-1β was marginally increased in DCL.
Figure 3. Macrophage chemoattractant protein (MCP)-1 expression and localization of dermal macrophages in serial sections of lesion of localized cutaneous leishmaniasis. MCP-1 mRNA expression (A) and MCP-1 immunoreactivity (B) correlate with dense infiltration of CD68 macrophages (C). Hair follicle (F) shown in 3 serial sections. In situ hybridization done with 35S-UTP-labeled antisense RNA probes (A) and immunohistology with MCP-1 antiserum and anti-CD68 monoclonal antibody using 3-step streptavidin-peroxidase method with 3-amino-9-ethyl-carbazole as substrate (B, C). Illumination: darkfield (A), brightfield (B, C). Bar = 50 µm.

Figure 4. In situ hybridization for macrophage chemoattractant protein (MCP)-1 mRNA (A, C) and macrophage inflammatory protein (MIP)-1α mRNA (B, D) expression in serial sections of localized cutaneous leishmaniasis (LCL; A, B) and diffuse cutaneous leishmaniasis (C, D) lesions using 35S-UTP-labeled antisense RNA probes. In LCL, basal keratinocytes (arrows) also express MCP-1 mRNA (A). Darkfield illumination. Bar = 100 µm.
On the other hand, in control (psoriasis) sections, high levels of IL-8 were detected mainly in the upper epidermis. Furthermore, no cross-hybridization between the highly abundant chemokines (MCP-1, MIP-1α) and the less abundant chemokines (RANTES, MIP-1β, I-309) was detectable despite their high homology. This finding coupled with those of the sense controls demonstrate that the hybridization was highly specific.

Discussion

A major characteristic of Leishmania-infected skin lesions is the massive infiltration with macrophages. During the course of infection, macrophages display multiple functions. They serve as host cells for the intracellular replication of parasites, as modulators of the specific immune activity by presenting parasite antigen to T cells, and as the ultimate mediators of the host response. Several reports have shown that the activation of antimicrobial effector functions in macrophages and, as a consequence, resistance or susceptibility of the host to Leishmania infections correlate with distinct patterns of cytokine production in the infected skin [29–31]. The present study provides the first evidence that self-healing LCL and progressive DCL, in addition to differences in lymphokine expression, have differential expression of monocyte-macrophage attractant chemokines.

The most conspicuous finding of this study was that MCP-1 and MIP-1α are reciprocally expressed in LCL and DCL skin lesions, respectively. In LCL lesions, MCP-1 was the prevalent chemokine, with massive expression in ~6% of all dermal cells and in basal keratinocytes, whereas only moderate levels of MIP-1α were detected. In DCL lesions, MIP-1α expression predominated and MCP-1 levels were much lower.

Our data may explain the massive accumulation of macrophages in both LCL and DCL, since MCP-1 and MIP-1α exert comparably strong chemotactic activities for monocytes and macrophages [32, 33]. However, the different characteristics of LCL and DCL lesions, with a low frequency of infected macrophages in LCL and a massive load of parasitized macrophages in DCL lesions, strongly suggest that the lesional macrophages in LCL and DCL exhibit different effector activities. Analogous findings have been reported for lepromatous and tuberculoid leprosy [34]. Therefore, it is tempting to speculate that MCP-1 and MIP-1α attract macrophages at different stages of activation and hence with different effector activities or, alternatively, display different stimulatory activities in addition to their chemotactic properties. Recent data show that chemokines, in addition to their chemotactic functions, exert a plethora of other activities [33, 35]. MCP-1 is much more potent than MIP-1α in inducing the release of granule enzyme N-acetyl-β-D-glucosaminidase from monocytes [33]. Even more important, MCP-1, but not MIP-1α, stimulates oxidative burst activity in macrophages, which is an important mechanism for killing intracellular amastigotes [36, 37]. Indeed, data from our laboratory show that treatment of Leishmania-infected human macrophages with MCP-1, but not MIP-1α, enhances their ability to kill the intracellular parasite (unpublished data). Taken together, our data strongly support the notion that MCP-1, in contrast to MIP-1α, not only accounts for the accumulation of dermal macrophages in leishmaniasis but contributes directly to the elimination of the parasite load via the stimulation of macrophages. The inverse relationship of MCP-1 expression in LCL and DCL may thus be related to the different disease courses.

Other studies have shown that certain macrophage attractant chemokines (MCP-1, MIP-1α and -1β) and the neutrophil attractant IL-8 also display chemotactic properties for various lymphocyte subsets [20, 21, 38–40]. Therefore, we were interested in the role of these cytokines with respect to lesional lymphocyte recruitment. Our data revealed a lower number of CD3 cells and a higher ratio of CD8:CD4 cells in DCL compared with LCL and are in agreement with a previous study [7]. Thus, the possibility exists that differences in chemokine patterns are responsible for the different lymphocyte profiles in LCL and DCL. MIP-1α preferentially stimulates migration of CD8 cells in vitro [39], whereas MCP-1 appears to induce migration of T cells of the memory phenotype without any preference for CD4 or CD8 cells [38]. Assuming that these selective chemotactic properties are also valid in vivo, the finding that MCP-1 expression is much higher in LCL may explain the higher number of T cells in LCL than in DCL lesions. Furthermore, the high levels of MIP-1α in DCL lesions may interfere with the local activation of T cells because this chemokine can inhibit anti-CD3–mediated T lymphocyte proliferation and production of IL-2 [41].

Even though the radioactive hybridization technique did not allow the simultaneous detection of leukocyte-specific markers on mRNA-positive cells, the hybridization pattern indicated that the highly abundant chemokines MCP-1 and MIP-1α are mainly produced by inflammatory cells and not by resident cells (e.g., endothelial cells and fibroblasts). In LCL, not only macrophages and T lymphocytes but also keratinocytes produce MCP-1. The correlation between MCP-1 production in basal keratinocytes and macrophage infiltration has been clearly shown in psoriasis lesions [16] and supports the notion that, in LCL, keratinocytes actively participate in the inflammatory process. This may also explain why the inflammatory infiltrate is primarily in the upper part of the dermis in LCL, whereas in DCL lesions, where basal keratinocytes do not express MCP-1, the inflammation is less concentrated in the upper dermis and extends to the lower dermis.

The results presented here are thought to be the first comprehensive data on chemokine expression in vivo in an infectious disease. Taken together, the chemokine profiles detected in LCL and DCL help explain the massive lesional recruitment of mononuclear cells, particularly macrophages, and the different composition of the lymphocyte subsets in the lesions. Furthermore, they suggest that chemokines, in addition to their chemotactic properties, may modulate the effector functions of macrophages and lymphocytes. The predominance of MCP-1 in LCL must be regarded as beneficial, whereas the prevalence of MIP-1α in DCL correlates with a progressive course of disease.
Figure 5. Macrophage inflammatory protein (MIP)-1α expression and localization of dermal macrophages in serial sections of localized cutaneous leishmaniasis lesion. MIP-1α mRNA expression (A) and MIP-1α immunoreactivity (B) correlate with dense infiltration of CD68 macrophages (C). In situ hybridization used 35S-UTP-labeled antisense RNA probes (A). Immunohistology done with anti-MIP-1α antiserum and anti-CD68 monoclonal antibody using 3-step streptavidin-peroxidase method with 3-amino-9-ethyl-carbazole as substrate (B, C). Illumination: darkfield (A), brightfield (B, C). Bar = 50 μm.
These findings may have important implications for the development of novel therapeutic strategies.

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References


