INFECTION AND IMMUNITY, Aug. 2002, p. 4177–4184 Vol. 70, No. 8
0019-9567/02/$04.00+0 DOI: 10.1128/IAI.70.8.4177–4184.2002

Leishmania Promastigotes Release a Granulocyte Chemotactic Factor and Induce Interleukin-8 Release but Inhibit Gamma Interferon-Inducible Protein 10 Production by Neutrophil Granulocytes

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Received 10 January 2002/Returned for modification 2 April 2002/Accepted 30 April 2002

Recent data from our laboratory suggest that neutrophil granulocytes (polymorphonuclear leukocytes [PMN]) can serve as host cells for *Leishmania major* in the early phase of infection. In line with these findings, an early influx of PMN to the infected tissues was shown by others to be associated with susceptibility to infection with *L. major*. The mechanisms underlying the initial PMN recruitment to the site of infection is poorly understood. In the present study we investigated whether *Leishmania* can influence PMN migration. Supernatants of *Leishmania* promastigotes were tested for their chemotactic activity using an in vitro chemotaxis assay. All *Leishmania* species tested (*L. major*, *L. aethiopica*, and *L. donovani*) displayed a marked chemotactic effect on human PMN. However, no effect on the migration of macrophages and NK cells was observed. Checkerboard analysis revealed that the observed PMN migration was due to chemotaxis rather than chemokinesis. Most of the chemotactic activity was found in fractions containing molecules with sizes between 10 and 50 kDa. Pretreatment of PMN with *N*-formyl-methionyl-leucyl-phenylalanine blocked the chemotactic activity of *Leishmania* supernatants up to 75%. In addition, we found that leishmanial contact induced the release of interleukin-8 (IL-8) and inhibited the production of gamma interferon-inducible protein 10 (IP-10) by PMN. These data suggest that infection with *Leishmania* promastigotes leads to PMN accumulation via the production of a chemotactic factor by the parasites, and this effect is amplified by the induction of IL-8 production in PMN. On the other hand, the inhibition of IP-10 production can lead to prevention of NK cell activation.

Leishmaniosis is initiated by the bite of an infected sand fly and the deposition of the promastigote form of the parasites in the skin of the vertebrate hosts (for a review, see reference 21). *Leishmania* sp. promastigotes are obligate intracellular pathogens which are rapidly destroyed in the extracellular tissue environment (17). Therefore, recruitment of "shielding" host cells is crucial for the survival of the parasites (21). In previous studies we and others demonstrated that neutrophil polymorphonuclear leukocytes (PMN) are attracted to the site of infection within 24 h (9, 24). Since recruitment of macrophages, the ultimate host cell for *Leishmania*, requires 2 to 3 days, the rapid attraction of PMN suggested that these cells may serve as host cells for *Leishmania*. Indeed we demonstrated that *Leishmania* promastigotes could infect neutrophil PMN and survive intracellularly in these cells (10). More-recent data from our laboratory demonstrated that infection with *Leishmania* extends the life span of PMN via the inhibition of their spontaneous apoptosis (1). These data suggest that neutrophil PMN can serve as host cells for *Leishmania* in the early phase of infection. This is in line with the observation that an early wave of PMN was shown to be associated with the development of a disease-promoting Th2-response in mice susceptible to *L. major* (25).

Since the recruitment of PMN appears to be beneficial for the survival of *Leishmania* in the infected tissues, we asked whether the parasite could actively influence PMN migration. Pathogenic microorganisms have devised several different strategies to influence the recruitment of leukocytes. Viral pathogens can directly interfere with leukocyte recruitment by the production of chemokine homologues, chemokine receptor homologues, or chemokine antagonists in order to avoid the recruitment of effector cells of the immune system (3). Bacterial pathogens can release chemotactic peptides like *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) to directly attract PMN (4, 6), protozoa such as *Toxoplasma gondii* were shown to release factors chemotactic to human leukocytes (16), and the release of PMN and monocyte-specific chemotactic molecules by different strains of *Leishmania* promastigotes has been suggested (22).

In light of the new role of PMN as host cells for *Leishmania*, in the present study we investigated the capacity of different strains of *Leishmania* promastigotes to modulate leukocyte recruitment early after infection. We showed that *Leishmania* promastigotes can induce a direct chemotaxis of PMN but not NK cells and monocytes. In addition leishmanial contact was found to influence the capacity of PMN to release chemokines.

MATERIALS AND METHODS

Preparation of human peripheral blood neutrophils, monocytes, and NK cells. Buffy coat obtained from healthy adult volunteers was diluted in sterile phosphate-buffered saline and layered on a Histopaque gradient consisting of Histopaque1077 (top) and Histopaque1119 (bottom) (Sigma-Aldrich Chemie, Deisenhofen, Germany) and centrifuged for 5 min at 300 × *g* followed by 15 min at 800 × *g* at room temperature without a break. The interphase between blood and

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Histopaque1077, consisting mainly of mononuclear cells, was used for isolation of monocytes and NK cells using magnetic cell sorting and CD14 microbeads or an NK-cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's protocol. The enriched cell populations contained 85 to 90% monocytes or NK cells as demonstrated by fluorescence-activated cell sorting analysis (not shown).

The PMN-rich interphase between Histopaque1071 and Histopaque1119 was collected and washed twice in RPMI 1640 medium (Gibco Laboratories, Eggenstein, Germany), and the cells were further fractionated on a discontinuous Percoll (Pharmacia, Uppsala, Sweden) gradient consisting of layers with densities of 1,105 g/ml (85%), 1,100 g/ml (80%), 1,093 g/ml (75%), 1,087 g/ml (70%), and 1,081 g/ml (65%). After centrifugation for 25 min at 800 × *g*, the interface between the 80 and 85% Percoll layers was collected and washed twice in RPMI 1640. All procedures were carried out at room temperature. The purity of PMN achieved by this isolation technique was always above 99% as determined microscopically after May-Gruenwald-Giemsa staining of cytocentrifuge (Shandon, Pittsburgh, Pa.) slides. The viability of cells was >98% as assessed by trypan blue dye exclusion.

***Leishmania* culture and LCF preparation.** *L. major* (MHOM/IL/81/FEBNI), *L. aethiopica* (MHOM/ET/72/L100), and *L. donovani* (MHOM/ET/67/HU3) promastigotes were cultured at 26°C for 8 days in RPMI 1640 medium (Gibco Laboratories) supplemented with 5% FCS (Seromed-Biochrom, Berlin, Germany) in a humidified atmosphere containing 5% CO_2 until they reached the stationary phase. Subsequently, promastigotes were collected and/or parasite-free supernatant was obtained by centrifugation (10,000 × *g* for 30 min). Parasite-free supernatants of *L. aethiopica* were subsequently lyophilized, and the resultant lyophilisate is referred to as *Leishmania* chemotactic factor (LCF) throughout the text.

Biochemical characterization of LCF. LCF samples were separated by size using the Amicon filtration technique. Using appropriate membranes, fractions containing molecules with sizes of either <10 kDa, sizes between 10 and 50 kDa, or sizes larger than 50 kDa were prepared. The fractions were then treated under different pH conditions. Neutral pH was changed to a pH of <2.0 by adding 25% HCl or to a pH of >10.0 by adding 10 N NaOH (for 15 min), after which the pH was neutralized again. In addition, fractions were heat treated at 100°C for 30 min or treated with proteinase K (200 μg/ml for 30 min at 37°C; Boehringer Mannheim Corp., Indianapolis, Ind.) or trypsin (1 mg/ml for 15 min at room temperature; Sigma-Aldrich Chemie). All treatments were followed by centrifugation at 10,000 × *g* for 30 min. Supernatants were collected and tested for chemotactic activity.

Coincubation of *Leishmania* promastigotes and PMN. In order to test for interleukin-8 (IL-8) release, 0.5×10^7 PMN were coincubated with *L. major* promastigotes at a parasite-to-PMN ratio of 5:1 at 37°C in a humidified atmosphere containing 5% CO_2 in a volume of 1 ml of complete medium (RPMI 1640 medium [Gibco Laboratories] supplemented with 20% heat-inactivated fetal calf serum, 50 μM 2-mercaptoethanol, 2 mM L-glutamine, 10 mM HEPES, penicillin [100 μg/ml], and gentamicin [160 μg/ml], all from Seromed-Biochrom). Supernatants were collected after 18, 42, and 66 h of incubation. In order to test for the ability of PMN to release gamma interferon (IFN-γ)-inducible protein 10 (IP-10) similar coincubations were performed with the addition of recombinant IFN-γ (100 U/ml; Prepro-Tech Inc., Frankfurt, Germany).

Chemokine concentrations in the supernatants were assessed by IL-8 (detection limit, 25 pg/ml) and IP-10 (detection limit, 5 pg/ml) enzyme-linked immunosorbent assay (ELISA) kits (DuoSet ELISA Development System [R&D Systems, Wiesbaden, Germany] and Biosource [Camarillo, Calif.] ELISA kit, respectively).

Chemotaxis assay. Chemotaxis assays were performed with highly purified human PMN, monocytes, and NK cells. As positive controls for chemotaxis, IL-8 (0.2 to 200 ng/ml; Prepro-Tech Inc.) and fMLP (1 μM; Sigma-Aldrich) were used for PMN; macrophage inflammatory protein 1 (MIP-1β; 50 ng/ml and monocyte chemoattractant protein 1 (MCP-1; 100 ng/ml) were used for monocytes; and MCP-1 (100 ng/ml) and IP-10 (50 ng/ml) (all from Prepro-Tech Inc. except as specified) were used for NK cells. The *Leishmania* samples and/or chemokines were placed in the lower well of a 24-well Transwell plate (Costar, Bodenheim, Germany) in a volume of 600 μl. PMN, monocytes, and NK cells were suspended at 6×10^6/ml in complete medium and added to the upper chamber in a volume of 100 μl. A polycarbonate filter with a pore size of 3 μm separated the two chambers. The chambers were incubated for 90 min at 37°C in a humidified air atmosphere containing 5% CO_2. The number of cells that migrated into the lower chamber was determined. PMN were quantified using a glucuronidase assay (13). In short, cells in the lower compartment were lysed in 0.1% Triton X-100, and glucuronidase enzymatic activity was measured by using *p*-nitrophenyl-glucuronide (Sigma-Aldrich Chemie) as a substrate. The number of migrated cells was calculated from a standard curve obtained with lysates of known numbers of PMN. Control experiments showed that pretreatment of PMN with chemokines did not affect subsequent measurement of endogenous enzymatic activity in the cell lysates (not shown).

Migration of monocytes and NK cells was quantified by microscopically counting the cells in the lower well under a high-power field magnification. All assays were performed in triplicate. The chemotactic index (CI) was calculated by dividing the number of cells that migrated towards a stimulus by the number of cells that migrated in medium alone.

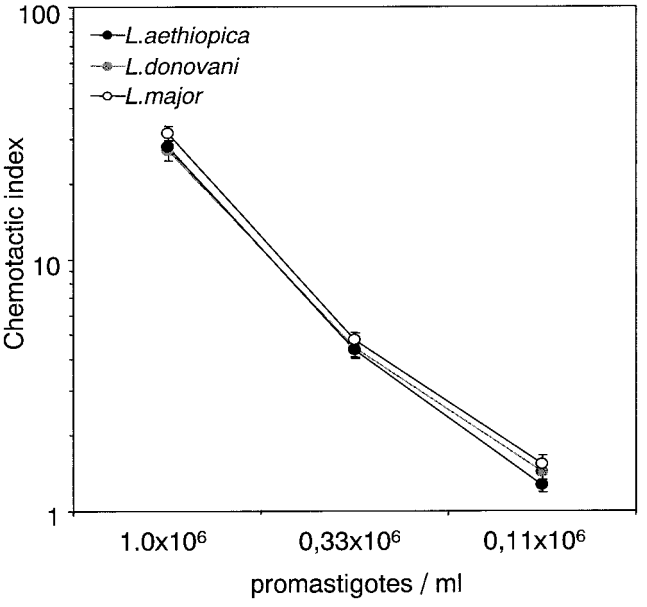

FIG. 1. Viable *Leishmania* promastigotes induce a dose-dependent migration of PMN. Viable promastigotes of *L. aethiopica*, *L. donovani*, and *L. major* were placed into the lower chamber of Costar Transwell plates and tested for their chemotactic activity for PMN in a 90-min migration assay. Migration of PMN was quantified using a glucuronidase assay. Migration is depicted as a CI: specific migration/migration towards medium. The figure shows the mean values ± the standard error of the mean (error bars) of duplicate assays for each condition obtained from three independent experiments.

RESULTS

***Leishmania* promastigotes release a chemotactic factor (LCF) for PMN.** Viable promastigotes of *L. major*, *L. donovani*, and *L. aethiopica* were tested for the release of chemotactic molecules. Live parasites induced a dose dependent migration of PMN (Fig. 1). No significant differences in chemotaxis induction was observed between the various *Leishmania* species.

Having observed that *Leishmania* promastigotes release a chemotactic factor, supernatants of parasites cultures were tested for the presence of chemotactic molecules. Parasitic supernatants of all three species induced a dose-dependent PMN migration (Fig. 2). IL-8, a known chemotactic factor for PMN, was used as a positive control. Migration of PMN towards the LCF was comparable with the migration towards IL-8 (Fig. 2). Since no significant differences between the chemotactic effect of *L. major*, *L. donovani*, and *L. aethiopica* was detected, for practical reasons we continued our experiments using a lyophilisate prepared from parasite-free supernatant of *L. aethiopica* cultures. When diluting the lyophilisate (LCF)

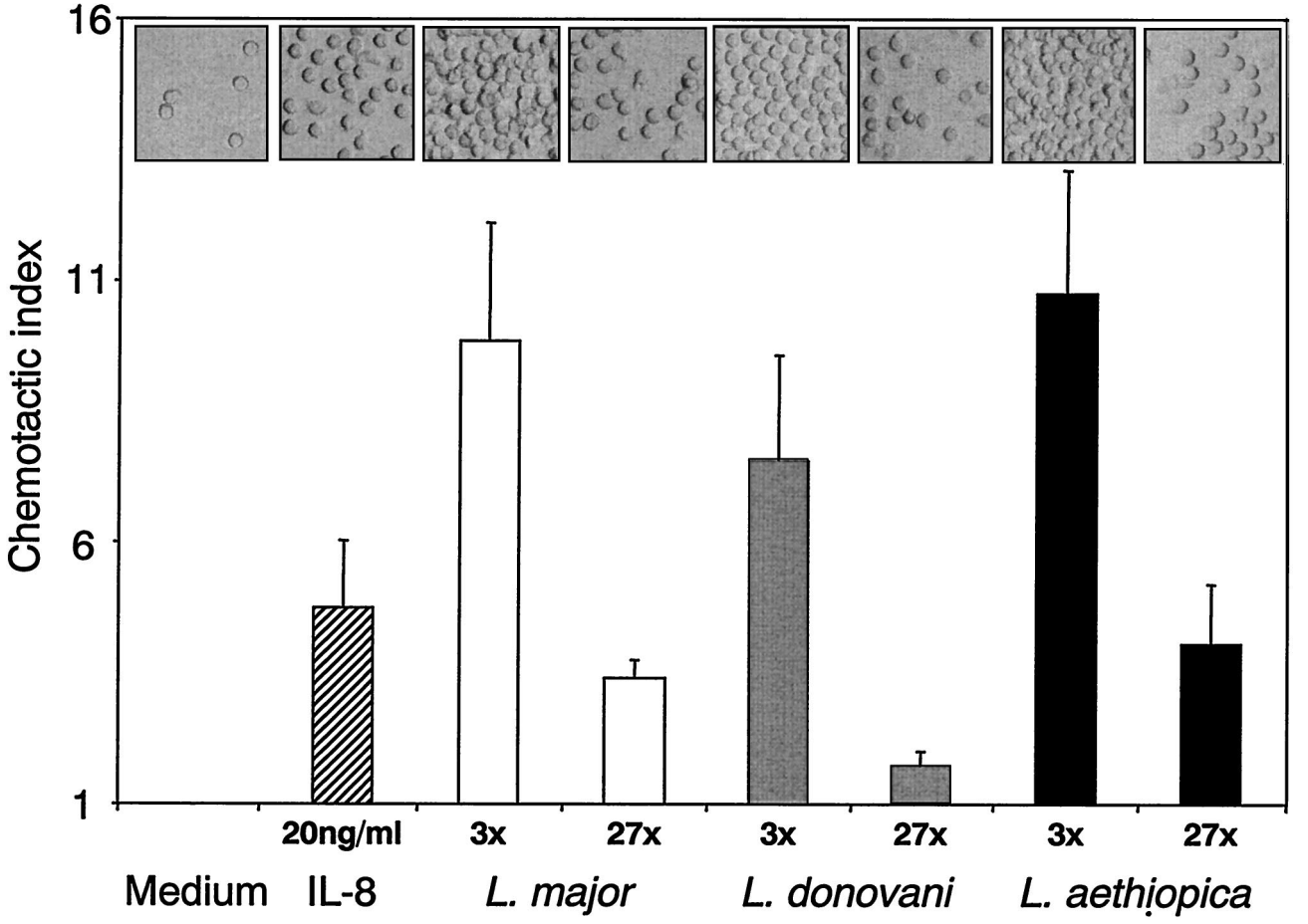

FIG. 2. Supernatants of *Leishmania* promastigote cultures contain a chemotactic factor (LCF) for PMN. The chemotactic activities of different dilutions of supernatants taken from *L. aethiopica* (filled bars), *L. donovani* (gray bars), and *L. major* (open bars) promastigote cultures were assessed as described in Materials and Methods. Recombinant IL-8 (20 ng/ml [hatched bars]) was used as a positive control. Results are depicted as CI. The figure shows the mean values + the standard errors of the means (error bars) of duplicate assays for each condition obtained from three independent experiments. Above each bar is shown a picture visualizing PMN recruitment to the bottom of the Transwell system. The pictures are representative of three independent experiments.

back to its original volume, chemotactic activity was equal to that of the original parasite-free supernatant (data not shown).

LCF attracts PMN via chemotaxis rather than chemokinesis. The LCF-induced PMN migration could be due to chemotactic or chemokinetic effects. The former means that the cell migration is a consequence of chemoattractive activity of LCF, whereas the latter implies the activation-associated migration of the cells in all directions. To differentiate between these two possibilities, a checkerboard analysis was performed (27). Various dilutions of LCF were applied to the upper and lower chambers, and PMN migration was assessed. As shown in Table 1, when LCF was added to the upper chambers, no significant migration was observed (CI ranging from 1.0 to 1.11 ± 0.3). In contrast, when LCF was added to the lower chamber, a dose-dependent migration was observed (CI ranging from 1.0 to 11.62 ± 2.1). Adding equal amounts of LCF in

TABLE 1. LCF attracts PMN via chemotaxis rather than chemokinesis[a]

LCF lyophilisate in lower chamber (mg/ml)	CI (mean ± SEM) when the indicated concn (mg/ml) of LCF lyophilisate was in upper chamber				
	0	0.31	0.63	1.25	2.5
0	1.00 ± 0.0	1.10 ± 0.3	1.05 ± 0.3	1.11 ± 0.3	1.01 ± 0.2
0.31	2.12 ± 0.5	2.00 ± 0.2	2.50 ± 0.7	1.78 ± 0.5	1.95 ± 0.2
0.63	2.61 ± 0.5	2.49 ± 0.2	2.53 ± 0.9	2.49 ± 0.9	2.90 ± 0.3
1.25	5.04 ± 0.6	2.91 ± 0.2	3.38 ± 0.5	3.51 ± 0.8	3.73 ± 0.6
2.5	11.62 ± 2.1	8.44 ± 2.3	6.08 ± 1.3	5.40 ± 1.3	6.06 ± 1.1

[a] Various dilutions of LCF were applied to the upper and lower chambers, and PMN migration was assessed and depicted as a CI. The table shows results of duplicate assays for each condition obtained from three independent experiments.

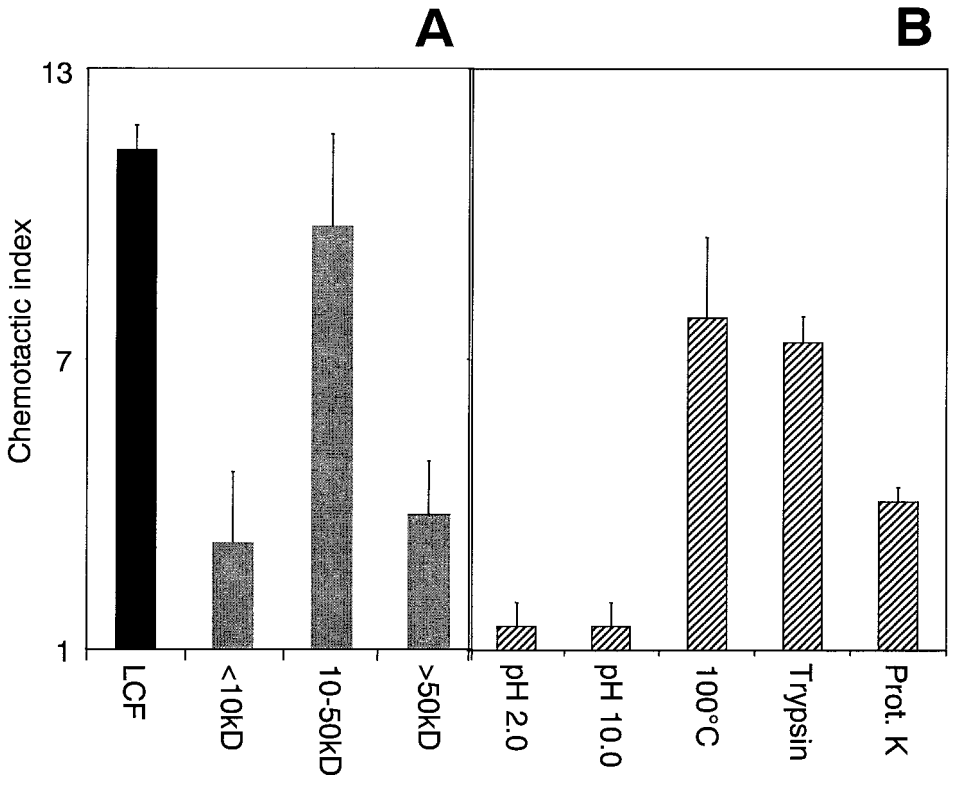

FIG. 3. LCF is between 10 and 50 kDa in size, heat stable, and proteinase K sensitive. (A) Unseparated LCF (black bar) and LCF fractions containing molecules smaller than 10 kDa, between 10 and 50 kDa, and larger than 50 kDa were tested at a concentration of 2.5 mg/ml for their chemotactic activity on PMN. (B) The effect of high and low pH, boiling at 100°C, and trypsin or proteinase K treatment on LCF chemotactic activity on PMN. Migration is depicted as CI. The figure shows the mean values + the standard errors of the means (error bars) of duplicate assays for each condition obtained from three independent experiments.

both the upper and lower compartments resulted in a significant reduction of PMN migration. These results indicate that the effect of LCF is chemotactic rather than chemokinetic.

LCF is heat stable, proteinase K sensitive, and between 10 and 50 kDa in size. For further characterization, LCF was separated by size using the Amicon filtration technique. Fractions were prepared containing either molecules <10 kDa, between 10 and 50 kDa, or larger than 50 kDa. When tested for PMN migration, most of the chemotactic activity was found in fractions containing molecules between 10 and 50 kDa in size (Fig. 3A).

To test the stability of LCF, the 10- to 50-kDa fraction was exposed to low- and high-pH conditions (pH < 2.0 and pH > 10.0, respectively), boiled at 100°C, and treated with trypsin or proteinase K. Treatment under both high- and low-pH conditions resulted in a complete loss of chemotactic activity. Boiling at 100°C did not affect the chemotactic activity, indicating that LCF is heat stable. Finally, trypsin treatment did not affect, whereas proteinase K decreased, the chemotactic activity of LCF (Fig. 3B).

FPR occupation on PMN inhibits LCF dependent chemotaxis. Chemotactic factors use specific receptors to induce cell migration. Therefore, we investigated the potential chemokine receptor usage of LCF on PMN. Known chemokine receptors on PMN are CXCR1; CXCR2 for IL-8; and the fMLP receptor (FPR). First, migration of PMN towards LCF, fMLP, and IL-8 was assessed (Fig. 4A). Consequently, migration experiments were carried out with PMN preincubated for 15 min with either IL-8 (Fig. 4B), fMLP (Fig. 4C), or LCF (Fig. 4D). As expected, preincubation with IL-8 inhibited the migration of PMN towards IL-8 (28). However, the chemotaxis of PMN towards LCF was not affected by this treatment (Fig. 4B). On the other hand, occupation of FPR by preincubation with fMLP inhibited the chemotaxis of PMN not only towards fMLP but also towards LCF up to 75% (Fig. 4C). Similarly, preincubation with LCF inhibited the migration of PMN towards both fMLP and LCF. These experiments suggest that LCF interacts with the FPR.

Neither viable *Leishmania* nor LCF induces the migration of NK cells and monocytes. *L. major* has been reported to induce the local recruitment of macrophages and NK cells after subcutaneous infection (8, 24). While macrophages are the major host cells of the parasites, NK cells have been shown to participate in the defense against the infection. The chemotactic activity of both viable *Leishmania* promastigotes and LCF on highly purified NK cells and monocytes was assessed. In the chemotaxis assays MCP-1 and IP-10 were used as positive controls for NK cell migration, and MIP-1β and MCP-1 were used as positive controls for monocyte chemotaxis.

No significant migration of NK cells towards either viable *L. aethiopica* promastigotes or LCF (CI 1.34 ± 0.2 and 1.36 ± 0.4, respectively) was observed, whereas control chemokines

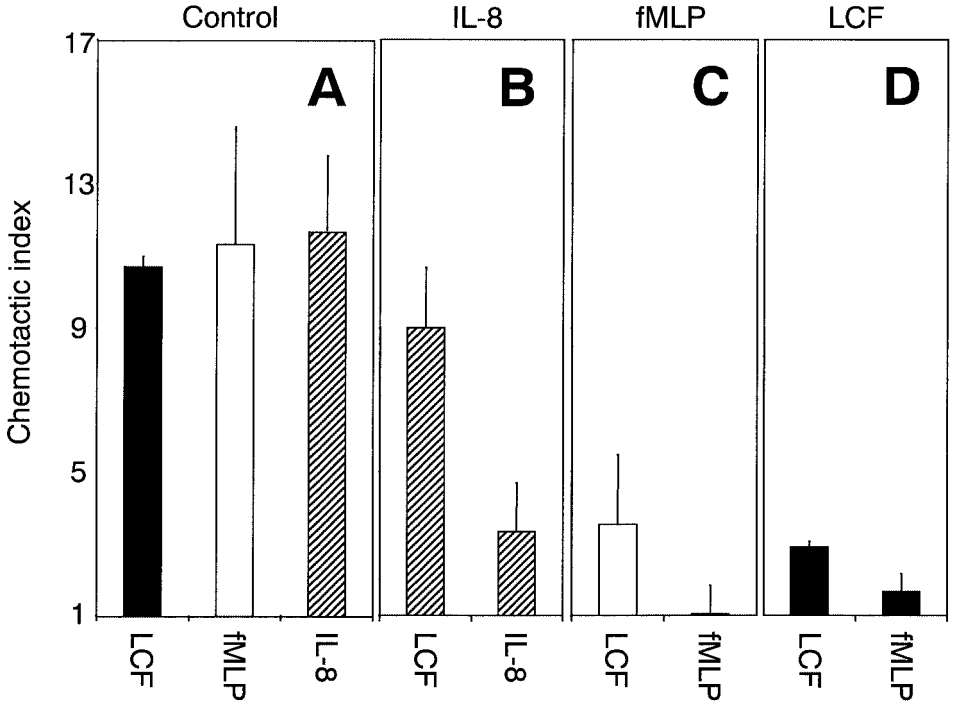

FIG. 4. LCF interacts with the FPR. (A) PMN migration towards LCF, fMLP, and IL-8. PMN were preincubated with either IL-8 (hatched bars) (B), fMLP (open bars) (C), or LCF (filled bars) (D), after which migration towards LCF, fMLP, and IL-8 was assessed. Migration is depicted as CI. The figure shows the mean values + the standard errors of the means (error bars) of duplicate assays for each condition obtained from three independent experiments.

MCP-1 and IP-10 induced the chemotaxis of these cells (CI 3.65 ± 1.4 and 3.30 ± 0.4, respectively) (Fig. 5). Similar results were found using *L. major* and *L. donovani* (data not shown).

Using monocytes as target cells, viable promastigotes did not induce their migration (Fig. 5). LCF led to a low-level chemotaxis of monocytes (CI, 1.52 ± 0.2), but this effect was minor compared to the migration towards MIP-1β and MCP-1 (CI, 2.10 ± 0.3 and 2.80 ± 0.3, respectively) (Fig. 5). These data indicate that *Leishmania* promastigotes do not secrete chemotactic factors for monocytes and NK cells.

Leishmanial contact induces release of IL-8 and inhibits the production of IP-10 by PMN. The data shown above suggest that *Leishmania* promastigotes, via the release of LCF, can recruit PMN to the infected tissue. PMN have been reported to produce several cytokines (19). Among others the release of the chemokines IL-8 and IP-10 by PMN has been demonstrated (1a, 5). The chemokine production by PMN is thought to affect the inflammatory process by recruiting or activating various leukocyte populations. Since the primary target cells of IL-8 are PMN, the production of this chemokine by inflammatory granulocytes appears to serve as an amplifying loop and attract more PMN to the site of inflammation. Additionally, IL-8 can prolong the life span of PMN (11).

The chemokine IP-10 is known to attract and activate NK cells (5) and IP-10 has been shown to be associated with resistance to *Leishmania* (26).

PMN were coincubated with *Leishmania* promastigotes, and culture supernatants were tested for production of IL-8. *L. major* promastigotes induced the release of IL-8 by PMN; the IL-8 content of culture supernatants reached a level of more than 2 ng/ml after 42 h. PMN cultured in the absence of *Leishmania* did not secrete IL-8 (Fig. 6A).

Coincubation with *L. major* did not induce the secretion of IP-10 by PMN. Stimulation of PMN with IFN-γ resulted in a significant release of IP-10 (Fig. 6B). However, *L. major* promastigotes eliminated the IFN-γ-induced IP-10 production of PMN nearly completely (Fig. 6B).

DISCUSSION

Previous studies from our laboratory and other laboratories revealed that PMN are the first leukocytes that appear at the subcutaneous site of *L. major* infection (15, 25). The mechanisms underlying the initial PMN recruitment to the site of infection are poorly understood. Here we have demonstrated that *Leishmania* promastigotes release a soluble chemotactic factor that attracts human PMN but not NK cells or macrophages. In addition, we showed that contact with *Leishmania* induces the IL-8 release by PMN but inhibits the ability of PMN to produce IP-10.

In a previous study sonicated *L. major amazonensis* was found to induce chemotaxis of PMN (18). We now have demonstrated that both viable promastigotes and supernatants of *Leishmania* cultures induced the migration of PMN in vitro. This suggests the active release of a chemotactic factor by *Leishmania.* Other studies revealed that apart from PMN, sonicated *Leishmania* could also attract monocytes (22). This is in contrast with our findings in which viable promastigotes and supernatant from *L. major*, *L. aethiopica*, and *L. donovani* specifically attracted PMN but not monocytes.

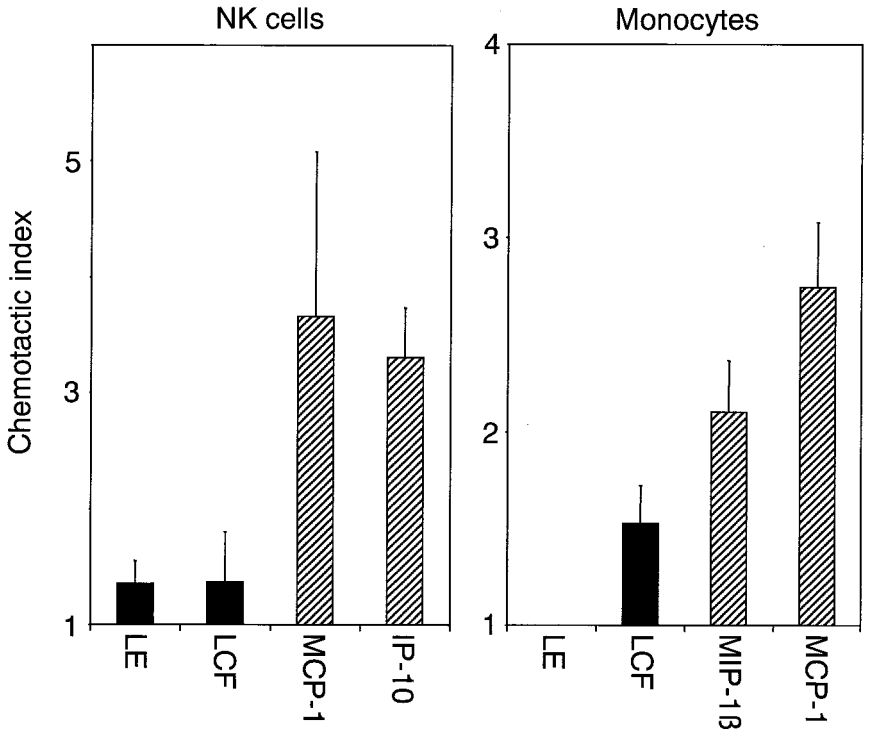

FIG. 5. *Leishmania* promastigotes and LCF are not chemotactic on NK cells and monocytes. Migration of purified NK cells and monocytes towards either viable *L. aethiopica* and LCF (filled bars) or control chemokines (hatched bars) was assessed. Migration was quantified by counting cells in the bottom wells after 90 min of incubation. The figure shows the mean values + the standard errors of the means of duplicate assays for each condition obtained from three independent experiments.

LCF appears to have characteristics similar to those of other known pathogen-derived PMN chemotactic factors. In both cultures and soluble extracts from *Spirometra erinacei*, chemotactic molecules of 14 kDa in size, specific for eosinophils and PMN, were detected (7). *T. gondii* has been shown to release PMN-attracting, heat-labile peptides in culture supernatant with M_w of 4.5 and 14 kDa (16). However, in contrast to our findings, soluble extracts from tachyzoites had negligible activity. Again a much larger chemotactic factor was found in supernatants from *Pasteurella haemolytica* cultures, in which a partially heat-resistant molecule larger than 100 kDa was described to be chemotactic for PMN and was suggested as an explanation for the rapid recruitment of PMN to the site of *P. haemolytica* infection (2).

Pretreatment with fMLP was found to inhibit the chemotactic activity of LCF up to 75%. Similarly, pretreatment with LCF inhibited the fMLP-induced migration of PMN. These findings suggest that either LCF binds the FPR or fMLP and LCF use a common pathway of PMN activation. LCF may contain fMLP-like peptide sequence motifs that can be recognized by the FPR. The FPR is known for binding microbial peptides containing fMLP sequences, this binding induces both a strong chemotaxis and the antimicrobial effect of the PMN (29). In addition, it was demonstrated that antimicrobial peptides of the cathelicidin family can use the FPR to attract and activate PMN (29). Similarly, a leucine zipper-like domain, T21/DP107, located in the amino terminus of the ectodomain of gp41, which is crucial to the formation of fusogenic configuration of the human immunodeficiency virus type 1 envelope protein gp41 was shown to attract and activate PMN through FPR (23). Therefore, the use of FPR-binding molecules appears to be a common strategy of several pathogenic microorganisms to influence PMN migration. Our present data suggest that *Leishmania* also releases an FPR-binding molecule to attract PMN.

Our present data suggest that *Leishmania* promastigotes can actively recruit PMN by the release of LCF. The subsequent parasite-induced secretion of IL-8 by PMN can function as amplifying loop resulting in the recruitment of more PMN to the site of *Leishmania* infection. LCF is, therefore, an important factor in the initial recruitment of PMN. The effect of LCF is then further amplified by PMN-derived chemokines. Indeed, studies in our laboratory revealed a rapid expression of KC and MIP-2 in the skin of mice infected with *L. major* (15). KC and MIP-2 are the functional homologues of IL-8 in mice (12).

The early influx of neutrophils into the infected tissue has been reported to be associated with the development of serious disease after *L. major* infection in mice (25). However, the mechanism by which PMN promote disease development has remained unclear. We have recently demonstrated the intracellular survival of *L. major* in PMN (10). PMN, therefore, can serve as host cells for *Leishmania* in the early phase of infection. The finding that *L. major* infection prolongs the life span of PMN by inhibiting the spontaneous apoptosis of these cells, represent a strong evidence to the role of PMN as host cells for the intracellular parasite *Leishmania*. Therefore, rapid recruitment of PMN is beneficial for the survival of parasites. Here we showed that *Leishmania* promastigotes release a chemotac-

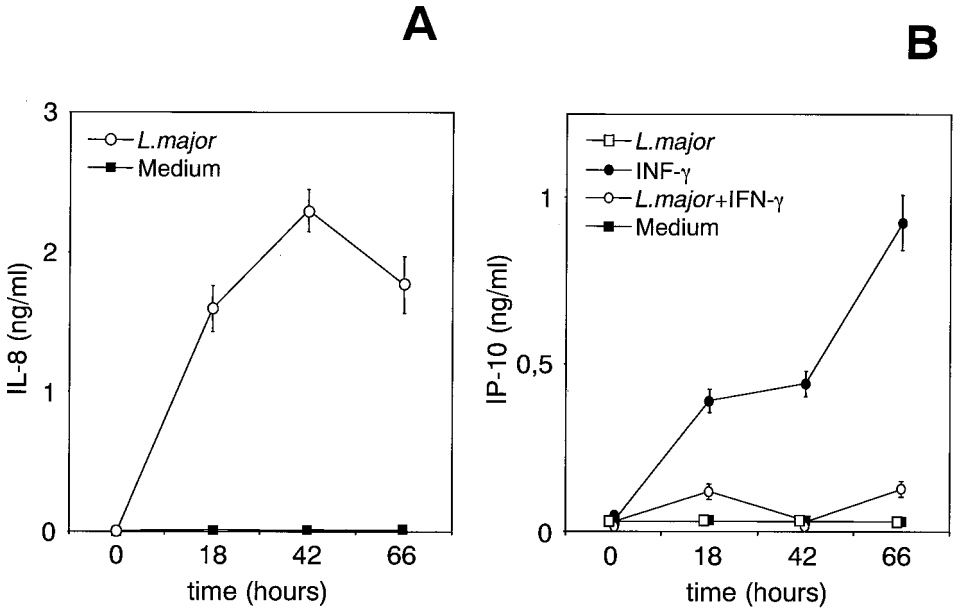

FIG. 6. *L. major* induces IL-8 release but inhibits IP-10 production of PMN. (A) PMN were coincubated with *L. major* or medium alone, and the IL-8 content of the supernatants was measured at the given time points by ELISA. (B) PMN were coincubated with *L. major*, recombinant IFN-γ, both *L. major* and IFN-γ, or medium alone, and IP-10 release was measured at the given time points by ELISA. The figure shows the mean values ± the standard errors of the means (error bars) of duplicate assays for each condition obtained from three independent experiments.

tic factor for PMN and, therefore, actively interact with PMN migration.

Natural killer cells play an important role in the defense to *Leishmania* infection (8). In the early phase of infection, activated NK cells, via the production of IFN-γ, promote the development a protective Th1-mediated cellular immune response. Depletion of NK cells was found to lead to increased susceptibility to *L. major* infection in mice (8, 20). One of the mediators of NK-cell recruitment and activation is the chemokine IP-10 (5). IP-10 can promote a Th1-response by acting directly on activated cells (20, 26). In accordance with these data, early expression of IP-10 was found to correlate with the resistant phenotype in a murine model of *L. major* infection (26). The fact that PMN are able to produce IP-10 suggests that inflammatory neutrophils can participate in the recruitment and activation of NK cells and Th1 cells, therefore, participate in the development of a protective immune response. We tested the IP-10 production of PMN after coincubation with *Leishmania* promastigotes. The results clearly show that the production of IP-10 by PMN is blocked in the presence of *Leishmania*. Although the mechanism of this action remains to be clarified, *Leishmania* appear to counteract the accumulation and activation of NK cells and Th1 cells in the infected tissue

Treatment of susceptible BALB/c mice with recombinant mouse IP-10 resulted in a significantly increased NK cell cytotoxic activity in the draining LN (26). Therefore, our finding that PMN are not able to produce IP-10 after leishmanial contact suggests an additional strategy for the parasite to escape immune defenses. *Leishmania* infection interferes with the modulatory functions of human PMN, altering NK cell activation and recruitment in the infected host. These data are in accordance with experiments demonstrating that the fungal pathogen *Cryptococcus neoformans* can inhibit IP-10 production to escape immune defenses (14).

Here we have shown that *Leishmania* promastigotes can regulate both the site-directed migration and the activation of cells of the innate immune response. Based on these findings, we suggest a twofold action of *Leishmania* parasites. On one hand, they release a chemotactic factor which directly attracts PMN, partially via the FPR on PMN. The infected PMN serve as an escape from the hostile extracellular environment. The production of IL-8 by PMN augments the enhanced influx of PMN serving as parasite "shelter." On the other hand, via the inhibition of IP-10 production, *Leishmania* can interfere with the development of a protective immune response.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 367 B10).

We thank E. Brandt and B. E. Schenk for helpful discussions concerning chemotaxis assays and A. Müller for providing the Amicon filter apparatus.

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Editor: J. M. Mansfield