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HUMAN RECOMBINANT MIGRATION INHIBITORY FACTOR ACTIVATES HUMAN MACROPHAGES TO KILL *Leishmania donovani*[1]

WEISHUI Y. WEISER,[2]* LU-ANN M. POZZI,* AND JOHN R. DAVID*†

*From the *Department of Medicine, Harvard Medical School, Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, MA 02115; and †Department of Tropical Public Health, Harvard School of Public Health, Boston MA 02115*

A recombinant form of the first lymphokine to be discovered, migration inhibitory factor (rMIF) was obtained from COS-1 cells transfected with a cDNA library from a human T cell hybridoma (6). rMIF has an amino acid sequence unrelated to that of any known protein. To learn more about the biology of MIF, we tested its ability to effect the survival of *Leishmania donovani* in macrophages. We found that rMIF activates blood monocyte-derived macrophages in vitro to suppress the growth of and kill these intracellular parasites. The anti-leishmanial effect (ranging from 50 to 77% reduction of parasites) is maximal when macrophages have been incubated with rMIF 48 to 72 h before infection and is similar to that seen with macrophages activated by IFN-γ. Of interest, whereas the activation of human macrophages by IFN-γ is inhibited by IL-4 and not enhanced by LPS, the activation by rMIF is enhanced by LPS but is not inhibited by IL-4. The data presented here demonstrate that rMIF is a potent activator of macrophages and is likely to be critical in cell-mediated immune host defenses.

The first lymphokine, MIF,[3] was discovered in 1966, when it was shown that Ag-stimulated lymphocytes produced a soluble factor that inhibited the migration of macrophage in vitro (1, 2). MIF-containing supernatants from stimulated lymphocytes were subsequently shown to alter macrophage function and enhance the killing of microorganisms and tumor cells (3–5). However, a purified or cloned lymphokine was required to demonstrate that these altered functions of macrophages were induced by MIF and not by another factor.

Recently, we used the in vitro inhibition of human blood monocyte migration to select a rMIF using a cDNA library from a T cell hybridoma expressed in COS-1 cells (6). This rMIF is a 12,000-Da polypeptide with an amino acid sequence unrelated to other known proteins. To learn more about the biology of MIF, we tested rMIF for its ability to inhibit the growth and kill the intracellular parasite, *Leishmania donovani*. We have also compared rMIF with another defined lymphokine, IFN-γ, known to enhance macrophage killing of *L. donovani* (7).

MATERIAL AND METHODS

Parasites. Promastigotes of *L. donovani* were obtained from the World Health Organization, Geneva, Switzerland, and maintained in vitro at 26°C in Schneider's *Drosophila* medium (GIBCO Laboratories, Grand Island, NY) (8). Amastigotes of *L. donovani* were obtained by stimulating THP-1 cells, a transformed human cell line from the monocyte lineage, with 100 ng/ml of PMA (Sigma Chemical Co., St. Louis, MO) for 18 h to induce differentiation and adherence. The nonadherent cells were removed, and the adherent cells were infected with promastigotes of *L. donovani* for 5 h. Then the uningested parasites were removed, and the cells were lysed by 0.01% SDS in HBSS to release the intracellular amastigotes, as previously described (9). The amastigotes and cell debris mixture were diluted 10-fold with PSGEMKA buffer (10) and the amastigotes were separated from the cellular debris by centrifugation (11). The viability of purified amastigotes was verified by allowing amastigotes to undergo transformation to the promastigote form at 26°C in Schneider's *Drosophila* medium supplemented with 20% FCS.

Cells. Human peripheral blood was obtained from healthy volunteers. Mononuclear cells isolated by Ficoll-Hypaque density centrifugation were suspended at 2.5×10^6 cells/ml in medium 199 (M. A. Bioproducts) containing 15% heat-inactivated FCS, 2 mM L-glutamine (GIBCO) and 5 μg/ml gentamicin; the suspension was seeded on top of four glass coverslips in a 35-mm petri dish (8). After 2 h of incubation at 37°C, 5% CO_2, the nonadherent cells were removed by washing, and the adherent cells were incubated for 7 to 10 days to obtain monocyte-derived macrophages.

Human rMIF and human rIFN-γ. rMIF, isolated by COS-1 cell expression screening of cDNA from a human T cell hybridoma line (T-CEMB) for MIF activity (6), was generated with the help of Genetics Institute, Cambridge, MA. rIFN-γ was purchased from Amgen (Thousand Oaks, CA.).

Infection of monocyte-derived macrophages. Promastigotes of *L. donovani* at the stationary growth phase or freshly isolated amastigotes were used to infect lymphokine-treated or untreated macrophages at parasite to cell ratios of 10:1 for promastigotes and 8:1 for amastigotes. These were applied to 35-mm petri dishes containing macrophages seeded on top of four coverslips. After 2 h of incubation at 37°C, the uningested parasites were removed by washing, and the cultures were reincubated in fresh medium. One coverslip from each dish was removed at 2 h, and three coverslips were removed at 48 h; these were washed in PBS and stained with Diff-Quick stain (American Scientific Products, McGaw Park, IL). The number of intracellular parasites was determined by microscopic examination of stained cells. From each coverslip, three or four randomly selected areas were examined; in each area, the number of parasites per 100 macrophages was counted. The average number of parasites ± SEM per 100 cells at 2 h (initial uptake) was obtained by dividing the total number of parasites from four random countings (400 macrophages) by four. The average number of parasites ± SEM per 100 cells at 48 h after infection was obtained by dividing the total number of parasites from three random countings of three coverslips (900 macrophages) by nine. In some experiments, the presence of living intracellular parasites was also measured by the incorporation of [^{3}H]TdR according to the method of Mauel et al. (12). Briefly, 48 h after infection, macrophages were lysed by 0.01% SDS in HBSS to release the surviving parasites. The parasites were cultured in me-

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[1] This work was supported by National Institutes of Health Grants AI 22801 and AI 22532.
[2] Address correspondence and reprint requests to Dr. Weishui Y. Weiser, Dept. of Medicine, Dept. of Rheum. and Immunol., Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115.
[3] Abbreviations used in this paper: rMIF, recombinant migration inhibitory factor; rGM-CSF, recombinant granulocyte-macrophage CSF.

dium containing 0.5 mCi of [^{3}H]TdR at 26°C for 20 h and the parasite growth then was measured by the incorporation of [^{3}H]TdR.

RESULTS

Killing of intracellular L. donovani by rMIF. Human monocyte-derived macrophages were incubated for 72 h with various dilutions of supernatants from COS-1 cells transfected with rMIF or mock-transfected controls. After incubation, the cells were washed and infected with promastigotes of *L. donovani*. After 2 h, the cultures were washed, and at this time comparable numbers of intracellular parasites in the rMIF-treated macrophages (239 ± 37) and control macrophages (224 ± 41) were found. After 48 h of infection, however, macrophages treated with rMIF contained many less ($p < 0.005$) intracellular parasites than macrophages treated with mock-transfected supernatants (Fig. 1*A*). Optimal anti-leishmanial effect of rMIF was seen after 48 to 72 h of incubation (Fig. 1*B*).

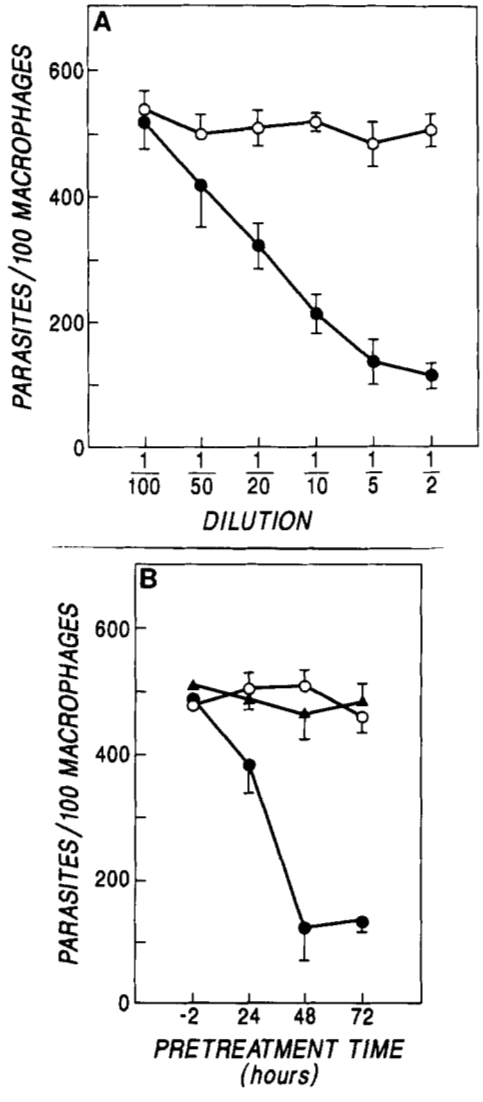

Figure 1. *A*, the dose response of rMIF. Human macrophages were incubated with rMIF (*closed circles*) or mock (*open circles*) transfected supernatants for 72 h. The cells were washed and infected with promastigotes of *L. donovani*. The number of parasites per 100 macrophages was determined 48 h after infection. *B*, time course of rMIF-induced anti-leishmanial activity. Human macrophages were incubated with rMIF (*closed circles*), mock (*open circles*), and medium (*closed triangles*) for 24, 48, and 72 h followed by infection with *L. donovani* for 2 h. Macrophages were also incubated with rMIF 2 h after infection. The number of parasites per 100 macrophages was determined 48 h after infection.

Table I summarizes the results of 11 experiments. Human monocyte-derived macrophages were incubated with rMIF-containing and control COS cell supernatants diluted 1/10 for 72 h. After incubation, the cells were washed and infected with *L. donovani* promastigotes, the form that infects mammalian cells (experiments 1 to 9), or amastigotes, the form that persists in macrophages (experiments 10 and 11). After 2 h of incubation, comparable numbers of intracellular parasites were found in the rMIF-treated and control macrophages. In contrast, after 48 h of infection, intracellular parasites were markedly decreased in macrophages treated with rMIF as compared with macrophages treated with mock-transfected supernatants ($p < 0.0001$ in all 11 experiments).

The reduction of parasites in the rMIF-treated macrophages ranged from 50 to 77% when compared with controls at 48 h. In five experiments, 2, 4, 7, 9, and 10, there were significantly less parasites per 100 macrophages at 48 h than at 2 h indicating cytotoxicity of rMIF-activated macrophages for the parasites. The results of the other six experiments suggest that the macrophages were cytostatic for the parasites. Furthermore, it should be noted that in these experiments remnants of destroyed organisms were seen in many macrophages preincubated with rMIF; this was not to be seen in controls.

In additional control experiments, COS-1 cells were transfected with a mutated MIF cDNA containing a stop codon in the middle of the coding region (6). Supernatants from such COS-1 cells had no migration inhibitory activity and did not activate macrophages to kill *Leishmania* amastigotes (Fig. 2). Furthermore, macrophages that were infected before incubation with rMIF did not become activated, nor did IFN-γ activate previously infected macrophages (7).

To further assess rMIF induced anti-leishmanial activity by macrophages and to determine whether the *Leishmania* remaining in the activated macrophages were still alive, we measured the incorporation of [^{3}H]TdR by the parasites, according to the method of Mauel et al. (12). In this assay, the growth of surviving parasites in the macrophages is reflected by their incorporation of [^{3}H]TdR. As is shown in Figure 3, there is significant reduction in uptake of [^{3}H]TdR into amastigotes of *L. donovani* taken from macrophages cultured with rMIF. Similar results were found using promastigotes (Fig. 4). These studies confirm the cytostatic or cytotoxic effect of rMIF-treated macrophages on the intracellular parasite *L. donovani*.

Effect of rMIF and rIFN-γ on L. donovani in human monocyte-derived macrophages. IFN-γ has been shown to activate human monocyte-derived macrophages to kill intracellular *L. donovani*, with optimal results found after 48 to 72 h of treatment before infection. (7). IFN-γ has also been shown to have MIF-like activity (13). When comparing the anti-leishmanial effect of monocyte-derived macrophages incubated with rMIF or rIFN-γ, we found that media containing 10-fold diluted rMIF had anti-leishmanial activity similar to media containing 1000 U/ml of rIFN-γ (Table II). Of note, the degree of inhibition of macrophage migration caused by 1000 U of rIFN-γ was similar to that of 10-fold diluted rMIF (data not shown).

We and others have noticed that not all the intracellular

TABLE I

Human macrophages activated by rMIF are cytotoxic and/or cytostatic for promastigotes and amastigotes of L. donovani

Expt. No.	Parasites/100 Macrophages[a]				Reduction %[b]	p (M48/M2)[c]
	Control 2 h	Control 48 h	MIF 2 h	MIF 48 h		
1	239 ± 27	460 ± 17	220 ± 10	213 ± 31	54	0.7
2	205 ± 13	484 ± 19	256 ± 9	174 ± 16	66	0.003
3	108 ± 10	270 ± 11	107 ± 14	120 ± 6	56	0.031
4	229 ± 11	306 ± 12	267 ± 6	153 ± 10	50	0.0002
5	177 ± 12	455 ± 8	199 ± 7	191 ± 9	58	0.44
6	181 ± 10	388 ± 12	175 ± 5	182 ± 11	53	0.34
7	361 ± 25	801 ± 39	377 ± 17	195 ± 11	76	0.0002
8	188 ± 5	551 ± 14	178 ± 7	253 ± 15	54	0.002
9	191 ± 12	439 ± 20	195 ± 13	102 ± 12	77	0.004
10	227 ± 20	339 ± 13	209 ± 11	165 ± 11	51	0.017
11	107 ± 4	430 ± 18	114 ± 6	125 ± 11	71	0.2

[a] Monocyte-derived macrophages were incubated for 72 h with 10-fold dilution of rMIF or control supernatant. The cells were infected with promastigotes (experiments 1 to 9) or amastigotes (experiments 10 and 11) of *L. donovani*. The number of intracellular parasites/100 macrophages at 2 h and at 48 h after infection was determined as detailed in *Materials and Methods*.

[b] Reduction % in each experiment was derived by comparing number of parasites in rMIF-treated macrophages with number of parasites in the controls at 48 h. The significance was analyzed and $p < 0.0001$ was found in all 11 experiments.

[c] The fate of parasites in each experiment from MIF-treated cultures was determined by comparing the number of parasites at 2 h (initial uptake) and at 48 h after infection. Of the 11 experiments, 5 (experiments 2, 4, 7, 9, 10) showed significant decrease in the number of intracellular parasites from the 2-h cultures suggesting cytolysis. On the other hand, 6 showed no significant difference between 2 h and 48 h, but there is cytostasis when compared with the controls at 48 h after infection.

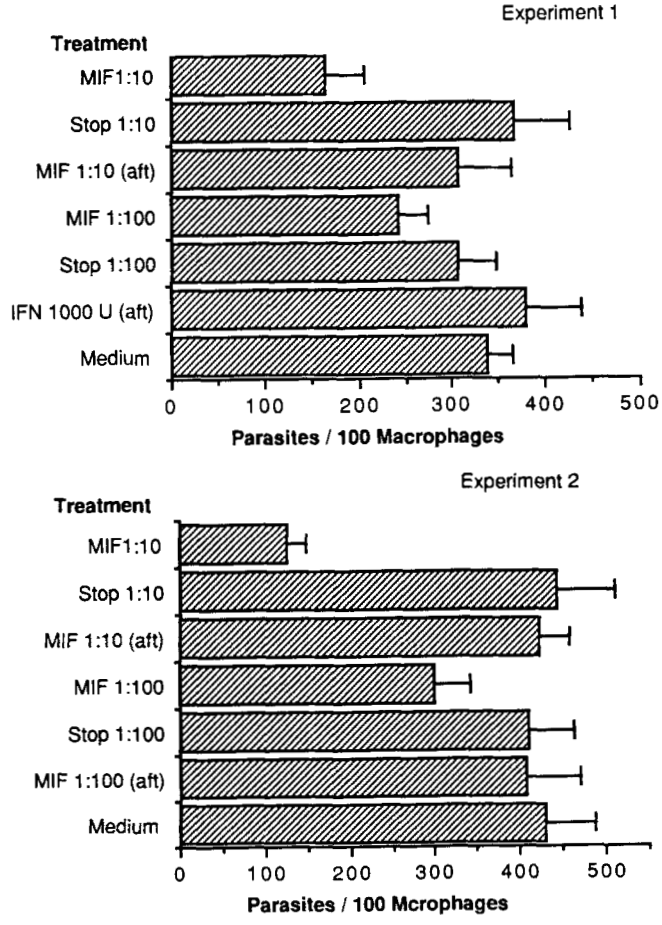

Figure 2. Cytotoxicity of rMIF-activated human macrophages against *L. donovani* amastigotes. Human macrophages were incubated with rMIF, rMIF containing a stop codon in the middle of the coding region (*stop*), or medium for 72 h followed by infection with amastigotes of *L. donovani*, which were obtained as described in *Materials and Methods*. Macrophages were also treated with rMIF after infection. The number of parasites per 100 macrophages was determined 48 h after infection. The data are presented as the mean ± SEM of nine separate quantifications of one experiment.

parasites are eradicated, even when optimal concentrations of rMIF, IFN-γ, GM-CSF, or other cytokines are used (7, 8, 14). Some residual parasites appear to remain insensitive to the treatment of macrophages with these lymphokines. In investigating whether the residual parasite population could be killed by treating monocyte-derived macrophages with a combination of rMIF and rIFN-γ, we found no significant enhanced killing of parasites when optimal doses, rMIF 1/10 and IFN-γ 1000 U/ml, were used together. (The numbers of *L. donovani* per 100 macrophages after 48 h were: experiment 1, control: 501 ± 40; rMIF: 239 ± 19; IFN-γ: 209 ± 25; combined: 243 ± 32; experiment 2, control: 477 ± 64, rMIF: 220 ± 35, IFN-γ: 239 ± 29, combined: 182 ± 17). In contrast, enhanced anti-leishmanial activity was observed ($p < 0.025$) when macrophages were incubated with a combination of the two lymphokines used at suboptimal doses, MIF 1:20, and IFN-γ 200 U/ml. (The numbers of *L. donovani* per 100 macrophages after 48 h were: experiment 1, control: 501 ± 40; rMIF, 377 ± 28; IFN-γ, 360 ± 20; combined: 198 ± 37; experiment 2, control: 477 ± 64; rMIF: 317 ± 20; IFN-γ, 415 ± 31; combined, 213 ± 17). Thus, other factors may be required to achieve total elimination of the parasites.

Anti-leishmanial activity of rMIF is further enhanced by LPS. Although bacterial LPS has been proposed as a second signal for macrophage activation in various experimental systems (15–17), we have previously demonstrated using human macrophages that both rGM-CSF and rIFN-γ are fully active under LPS-free conditions (8), and that the anti-leishmanial-inducing activity of these lymphokines is not enhanced by the addition of LPS (8). Also, we have shown that addition of 10 or 50 ng/ml LPS alone to macrophages exerted no anti-leishmanial effect. After processing and culturing human monocyte-derived macrophages under stringent LPS-free conditions, we investigated the effect of LPS on the potentiation of anti-leishmanial activity in rMIF-treated macrophages. In contrast to the observations with the other two lymphokines, we found that addition of 10 or 50 ng/ml of LPS to cultures of macrophages treated with optimal doses of

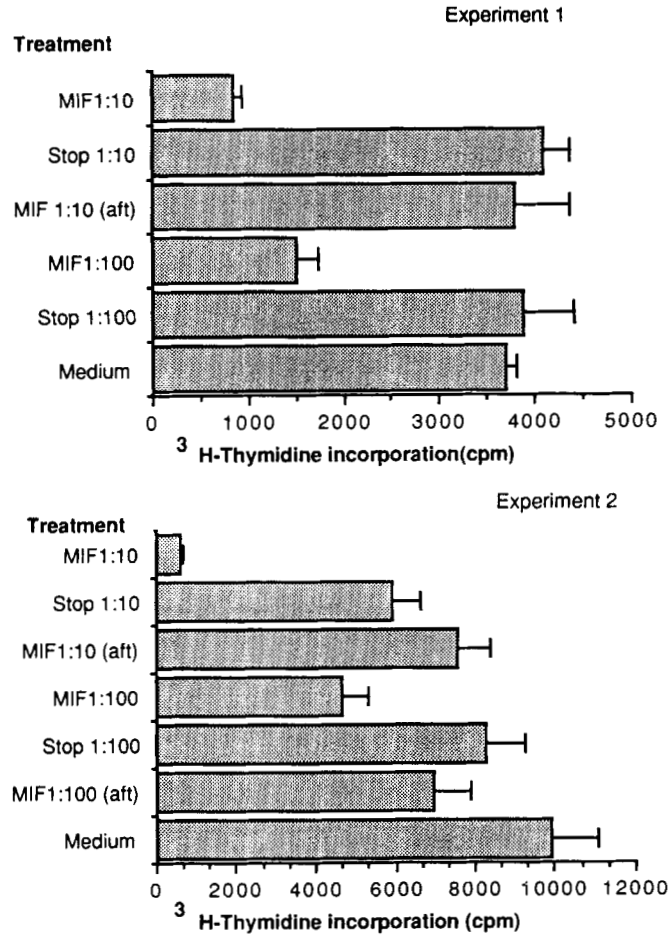

Figure 3. Cytotoxity of rMIF-activated human macrophages against *L. donovani* amastigotes as measured by the incorporation of [^{3}H]TdR. Human monocyte-derived macrophages were seeded in microtiter wells and incubated with rMIF, rMIF containing a stop codon in the middle of the coding region (*stop*), or medium for 72 h followed by infection with amastigotes of *L. donovani*, which were obtained as described in *Materials and Methods*. Macrophages were also treated with rMIF after infection. Forty-eight hours after infection, macrophages were lysed with 0.01% SDS for 45 min to release the intracellular parasites. The cultures were pulsed with [^{3}H]TdR for 20 h. The data represent mean ± SEM of six quantifications of one experiment.

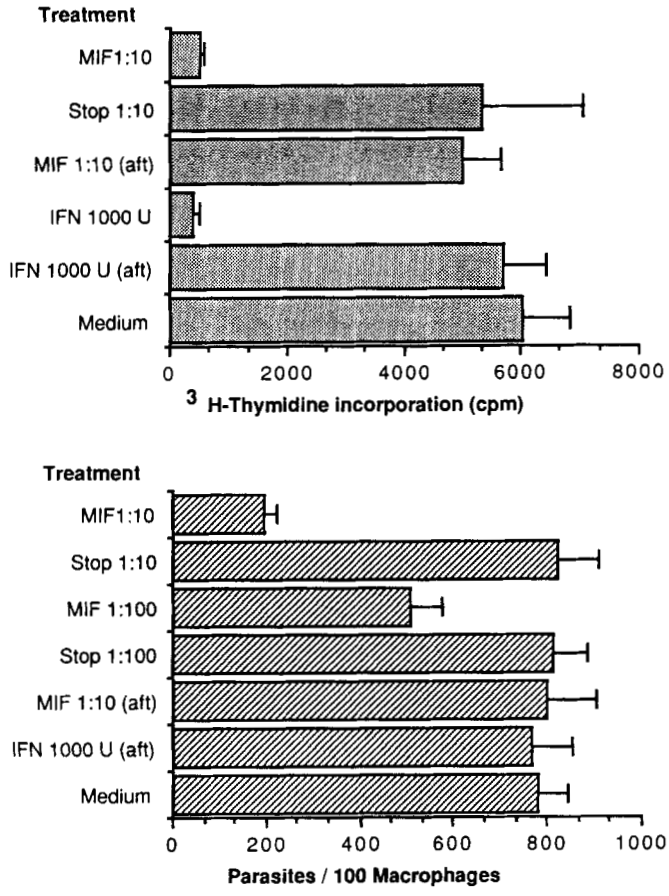

Figure 4. Cytotoxicity of rMIF-activated human macrophages against promastigotes of *L. donovani* as measured by the incorporation of [^{3}H] TdR (*upper panel*) and by counting of intracellular parasites (*lower panel*). Human monocyte-derived macrophages were incubated with rMIF, rMIF containing a stop codon in the middle of the coding region (*stop*), rIFN-γ, or medium for 72 h followed by infection with promastigotes of *L. donovani*. Human macrophages were also incubated with rMIF or IFN-γ after infection. To assess the uptake of [^{3}H]TdR, cells were lysed with 0.01% SDS 48 h after infection to release the intracellular parasites and the cultures were pulsed with [^{3}H]TdR as described in Figure 3. Counting of the intracellular parasites was performed as described in *Materials and Methods*. In the [^{3}H]TdR assay, the data represent mean ± SEM of six quantifications of one experiment whereas the data for the number of parasites per 100 macrophages represent mean ± SEM of nine separate quantifications of one experiment.

rMIF further increased their anti-leishmanial effect ($p < 0.025$) (Table III). This suggests that the mechanism of macrophage activation by rMIF differs from that of rIFN-γ or GM/CSF (7, 8).

rMIF induced anti-leishmanial activity is not abrogated by IL-4. IL-4, a B cell-stimulatory factor (18) has been shown to mediate multiple biologic functions in a variety of cell types (19–22). We have previously shown that IL-4 inhibits H_2O_2 production and anti-leishmanial capacity of human cultured monocytes mediated by IFN-γ (23). In addition, IL-4 also abolished GM-CSF-mediated anti-leishmanial capacity of macrophages and exacerbated the infection in human cultured monocytes (24). In mice, IL-4 has been observed in lesions of nonhealer mouse strains infected with *L. major* (25, 26) and activation of murine macrophage by IFN-γ from host-protective T cells is inhibited by IL-4 produced by disease-promoting T cells in leishmaniasis (27). Therefore, we investigated the effect of IL-4 on the anti-leishmanial activity of macrophages induced by rMIF. Under the same experimental conditions, IL-4 (32 to 1000 U/ml) was administered together with rMIF to monocyte-derived macrophages before infection by *L. donovani*. In two experiments, we found that the anti-leishmanial effect of rMIF was not affected by the addition of IL-4 (Fig. 5). On the other hand, in experiment 1, the anti-leishmanial effect of IFN-γ was abrogated by IL-4 as expected.

DISCUSSION

In this study, we have demonstrated that human rMIF, obtained from COS-1 cells transfected with MIF-specific cDNA, activates human monocyte-derived macrophages to inhibit the growth of and/or kill promastigotes and amastigotes of *L. donovani*. The activation of the macrophages by rMIF requires pretreatment and the maximal anti-leishmanial effect is seen after the macrophages have been preincubated with rMIF for ≥ 48 h. However, adding rMIF or IFN-γ to macrophages already infected with the parasites is not effective in inducing the infected macrophages to reduce the number of intracellular parasites.

Furthermore, we have shown that the activation of human macrophages by rMIF differs in several aspects

TABLE II

Antileishmanial activity of rMIF and rIFN-γ

Treatment[a]	No. of Parasites/100 Macrophages[b]							
	Expt. 1		Expt. 2		Expt. 3		Expt. 4	
	at 2 h	at 48 h	at 2 h	at 48 h	at 2 h	at 48 h	at 2 h	at 48 h
Mock[c] 1/10	239 ± 17	461 ± 39	205 ± 18	484 ± 29	108 ± 6	270 ± 6	229 ± 22	306 ± 8
MIF 1/10	220 ± 17	213 ± 56	256 ± 13	174 ± 29	107 ± 21	120 ± 5	266 ± 37	153 ± 9
IFN 1000 U/ml	243 ± 33	223 ± 46	199 ± 29	196 ± 57	129 ± 8	113 ± 7	251 ± 19	119 ± 14
Mock 1/20	229 ± 11	510 ± 15	196 ± 21	475 ± 31	119 ± 9	280 ± 16		
MIF 1/20	213 ± 8	367 ± 26	244 ± 31	312 ± 30	121 ± 17	210 ± 12		
IFN 200 U/ml	195 ± 14	405 ± 51	217 ± 15	400 ± 24	117 ± 15	219 ± 14		

[a] Monocyte-derived macrophages were treated as indicated above for 72 h. The cells were then infected with promastigotes of *L. donovani* for 2 h. Coverslips were removed at 2 h and 48 h after infection.

[b] The number of intracellular parasites per 100 macrophages at 2 h and at 48 h after infection was determined. The data derived from cultures treated with rMIF, rIFN-γ and mock were analyzed. There exists statistically a significant different on antileishmanial activity between cells treated with rMIF and mock as well as between rIFN-γ and mock ($p < 0.005$), whereas there exists no significant difference on antileishmanial activity between cells treated with rMIF and rIFN-γ ($p < 0.25$).

[c] Supernatant from COS cells transfected with MIF-cDNA containing a stop codon in the coding region was used in experiment 4.

TABLE III

LPS is able to enhance the antileishmanial activity of rMIF

Treatment[a]	No. of Parasites/100 Macrophages[b]			
	Expt. 1		Expt. 2	
	At 2 h	At 48 h	At 2 h	At 48 h
None	177 ± 14	455 ± 27	181 ± 16	388 ± 37
LPS 50 ng/ml	183 ± 21	470 ± 41	173 ± 25	372 ± 12
LPS 10 ng/ml	182 ± 10	468 ± 24	171 ± 11	365 ± 25
MIF 1/5	199 ± 27	191 ± 22	175 ± 33	182 ± 10
MIF + LPS 50 ng/ml	201 ± 31	105 ± 14	193 ± 37	116 ± 24
MIF + LPS 10 ng/ml	169 ± 29	126 ± 17	188 ± 17	114 ± 13

[a] Human monocyte-derived macrophages were pretreated as indicated above for 72 h. The cells were infected with *L. donovani* for 2 h. Coverslips were removed at 2 h and 48 h after infection. There is significant enhancement of activity by adding LPS to rMIF-treated cultures ($p < 0.025$).

[b] Average of four independent countings.

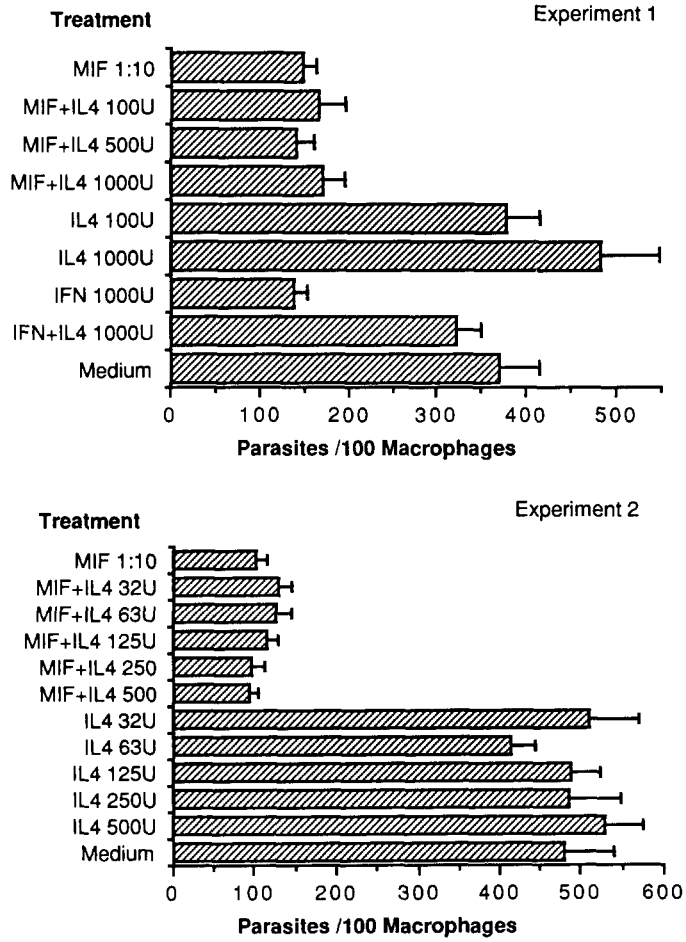

Figure 5. IL-4 does not inhibit activation by rMIF. Human monocyte-derived macrophages were pretreated as indicated above for 72 h. The cells were infected with promastigotes of *L. donovani* for 2 h. The number of parasites per 100 macrophages were determined 48 h after infection. The data represent mean ± SEM of nine separate quantifications of one experiment. No significant abrogation of antileishmanial activity is detected by adding IL-4 to rMIF-treated cultures ($p < 0.0001$).

from the activation induced by either IFN-γ or GM-CSF. Whereas the simultaneous addition of 10 or 50 ng/ml of LPS with the lymphokine does not enhance the anti-leishmanial activity induced by IFN-γ or GM-CSF (8), it does enhance the anti-leishmanial activity induced by rMIF. Likewise, the simultaneous addition of IL-4 with the activating lymphokine to human macrophages will inhibit the anti-leishmanial effect induced by IFN-γ (23) or GM-CSF (24), but does not diminish the anti-leishmanial activity induced by rMIF in the same assay system. These observations indicate that the mechanisms of activation induced by rMIF and its regulation leading to cytostasis or killing of *L. donovani* may not be identical to those of IFN-γ or GM-CSF.

In addition to activating human monocyte-derived macrophages to kill *L. donovani*, rMIF activates human macrophages to kill two human tumor cell lines: A375, a melanoma line, and K562, a myeloma line (28). Furthermore, in preliminary experiments, it has been observed that macrophages pretreated with rMIF contain reduced numbers of intracellular *Mycobacterium avium* compared with controls (Newman G., et al., personal communication). In the murine model, rMIF will activate mouse peritoneal macrophages to kill *L. major* in vitro (Titus R., et al., personal communication). Thus, macrophages activated by rMIF exhibit cytostasis and/or cytolysis of both intracellular and extracellular target cells.

Although the mechanism of rMIF-induced macrophage activation is unclear, we have found that human macrophages treated with rMIF elaborate significant amounts of TNF-α (20 to 35 U) into the culture medium as assessed by cytotoxicity to TNF-sensitive L929 cells. In contrast, macrophages treated with mock transfected supernatants that showed no anti-leishmanial activity did not elaborate any detectable TNF-α. TNF-α can mediate host

resistance against *L. major* infection (29–31) and can also activate mouse peritoneal macrophages to kill intracellular *L. major* (30, 31). Furthermore, endogenous TNF-α triggers the production of L-arginine-derived toxic nitrogen intermediates (32) that can kill intracellular *Leishmania* (12, 30, 33) and also appears to be involved in vivo resistance to this parasite (34). Of note, earlier reports indicated that H_2O_2 was the principal agent for killing *L. donovani* in lymphokine-activated human (35) or murine (36) macrophages. The relative role of toxic nitrogen intermediates in the killing of *L. donovani* by human macrophages is unknown and now is being investigated.

rMIF expressed in COS cells induces IL-1β and tumor necrosis factor and up-regulates the HLA-DR gene expression of human monocyte-derived macrophages (6). It has also been shown to have strong immuno-adjuvant-like activity (37). The data presented here demonstrate that rMIF is a potent activator of macrophages and is likely to be critical in cell-mediated immune host defenses.

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