INFECTION AND IMMUNITY, Sept. 1992, p. 3523–3527
0019-9567/92/093523-05$02.00/0

Vol. 60, No. 9

Granulocyte-Macrophage Colony-Stimulating Factor Increases the Infectivity of *Leishmania amazonensis* by Protecting Promastigotes from Heat-Induced Death

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Received 4 February 1992/Accepted 2 June 1992

We have studied the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) on the infectivity of promastigotes of *Leishmania amazonensis*, an obligate intramacrophage parasite. We measured the capacity of the promastigotes to infect macrophages after preincubation at different temperatures (28, 34, and 37°C) with recombinant murine GM-CSF, as well as the effect of an anti-murine GM-CSF antibody on the in vitro and in vivo infectivity of the parasite. GM-CSF increases the capacity of the promastigotes to infect cells when preincubated at 34 and 37°C, whereas the anti-GM-CSF antibody exerts the opposite effect: it decreases the internalization rate and the progression of infection in macrophage cultures and slows the growth of the lesion in infected BALB/c mice. Neither of the described effects were observed when the in vitro and in vivo infections were made with amastigotes. Promastigotes die in a time-dependent manner when incubated at temperatures higher than 28°C in the absence of GM-CSF. They are protected from this heat-induced death by incubation with the recombinant hormone. Our interpretation of these data is that the increase in the infectivity of promastigotes when incubated with GM-CSF at the temperatures at which infection occurs (34 and 37°C) is due to the larger number of surviving forms within the infecting population. The decrease in infectivity when they are incubated with the antibody is due to inhibition of the protection conferred by the GM-CSF produced by the macrophages during the in vitro and in vivo infections.

Leishmanias are eukaryotic parasites which infect cells of the reticuloendothelial system of several mammalian species including humans. They occur as flagellated promastigote forms in the intestinal tract of the insect vector, where they multiply extracellularly, and as amastigote forms, growing within the phagolysosome of macrophages (15). The dimorphic life cycle of these parasites requires the operation of biological mechanisms for survival in very different milieus. The differentiation from an extracellular promastigote into an intracellular amastigote certainly represents a dramatic example of biological adaptation to a hostile environment. During infection of susceptible mammals, most of the promastigotes die (9) as a consequence of adaptation to stresses, including heat shock. Therefore, only thermotolerant forms are really infective and are thus able to invade macrophages, transform into amastigotes, and replicate intracellularly. We have previously shown that granulocyte-macrophage colony-stimulating factor (GM-CSF), a hematopoietic growth factor produced by macrophages, endothelial cells, and activated T lymphocytes, acts as a growth factor for promastigotes of *Leishmania amazonensis* (1). Here we show that this hormone is able to protect promastigotes from heat-induced death and that one consequence of this effect is an increase in the infectivity of a population of promastigotes. Besides the very interesting biological phenomenon by which a vertebrate hormone protects an infective microorganism from death, our results may be an alternative explanation for data showing, on the one hand, correlations between GM-CSF and other hematopoietic factors and susceptibility to leishmanial infections in in vivo experimental models of cutaneous leishmaniasis (4, 5, 12) and, on the other, a stimulating effect of this hormone on the intramacrophagic killing of different *Leishmania* species (7, 10, 25).

MATERIALS AND METHODS

Mice. BALB/c female mice aged 6 to 8 weeks, raised in our own facilities, were used throughout these studies.

Parasites. *L. amazonensis* MHOM/BR/68/H-21 (H-21), kindly donated by the Wellcome Parasitology Unit, Evandro Chagas Institute, Belem, Brazil, and MPRO/BR/72M/1841 (LV-79), maintained by sequential passages in vitro and in vivo, were used. For in vitro growth, parasites were incubated at 28°C in screw-cap tubes containing brain heart infusion broth (37 g/liter; Difco Laboratories, Detroit, Mich.), hemin (0.01 g/liter dissolved in 2 M NaOH; Microbiologica, Rio de Janeiro, Brazil), and 10% heat-inactivated fetal calf serum (Culti-Lab, Campinas, Brazil). The passages were done with an inoculum of 10^6 parasites per ml every 7 days. For in vivo maintenance, the BALB/c mice were routinely injected in the footpads with 10^7 promastigotes. Amastigotes were collected by aspirating infected cells directly from the lesion into a syringe and plating them at 28°C in brain heart infusion broth. After differentiation to promastigotes, they were maintained as described above. Amastigotes for infecting cells in vitro were obtained by dissecting mouse lesions and disrupting the tissue with a glass pestle. The homogenized tissue was then centrifuged at very low speed (50 × *g*) to eliminate large debris. The amastigote-rich supernatant was collected in cold Hanks balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.) and subjected to two cycles of centrifugation at 500 × *g*. After each cycle, the supernatant crowded with small debris was dis-

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TABLE 1. Effect of rmGM-CSF on infectivity of promastigotes of *L. amazonensis* preincubated at different temperatures[a]

Preincubation temp (°C)	% of macrophages infected		Mean no. of parasites/infected macrophage		Infectivity index[b]	
	−rmGM-CSF	+rmGM-CSF	−rmGM-CSF	+rmGM-CSF	−rmGM-CSF	+rmGM-CSF
28	77.00	29.00	3.49	1.27	268.73	36.83
34	22.50	41.00	1.37	1.46	30.82	59.86
37	39.60	46.00	2.17	2.21	59.86	101.66

[a] Each incubation contained 3×10^6 parasites and was carried out for 4 h in duplicate cultures at the described temperatures.
[b] Differences between groups without and with GM-CSF were significant at $P < 0.01$, $P < 0.04$, and $P < 0.15$ for experiments at 28, 34, and 37°C, respectively.

carded, and finally the amastigotes were pelleted by centrifugation at 1,300 × *g*, counted in a hemocytometer, and resuspended to the desired concentration in RPMI 1640 (GIBCO)–2.5% fetal calf serum.

Reagents. Recombinant murine GM-CSF (rmGM-CSF) was kindly donated by Boehringher, Mannheim, Germany). Goat anti-murine GM-CSF antisera and affinity-purified antibodies were kindly donated by Jolanda Schreurs, DNAX, Palo Alto, Calif.

Assays for thermotolerance. For the direct measurement of promastigotes viability, we used a slightly modified version of the adaptation to *Leishmania* spp. (11) of the colorimetric assay described by Mosmann (17). This assay measures the cleavage by living, metabolically active cells, of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, Mo.). Stationary-phase promastigotes (10^7/ml) were incubated in 3-(*N*-morpholino) propanesulfonic acid (MOPS)-buffered saline (116.0 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM D-glucose, 30.0 mM MOPS) for different times at the desired temperatures, after which MTT cleavage was measured by using a multiwell scanning spectrophotometer (model 3550; Bio-Rad Laboratories, Richmond, Calif.) with a reference wavelength of 656 nm and a test wavelength of 595 nm.

Infectivity assays. In vitro infectivity was assayed by analyzing the number of amastigotes that infected macrophage monolayers. To this end, resident macrophages were collected from the peritoneal cavity of BALB/c mice, plated in round glass coverslips inside the wells of a 24-well culture dish (A/S Nunc, Roskilde, Denmark) at a concentration of 10^5 cells per coverslip in RPMI 1640 supplemented with 5% fetal calf serum, 2 mM L-glutamine (Sigma), and 10 μg of gentamicin (Schering S.A., Rio de Janeiro, Brazil) per ml. After 2 h of incubation at 37°C in an atmosphere of 5% CO_2, the medium was changed and the cells were left for an overnight incubation. Then promastigotes were added to the wells at different concentrations, and the cultures were further incubated at 34°C in a 5% CO_2 atmosphere. After 4 and 48 h of incubation, the coverslips were washed and stained with Giemsa stain, and the number of amastigotes per macrophage and the percentage of infected macrophages were assessed by light microscopy observation. An infectivity index was obtained by multiplying those two parameters of infection. The numbers obtained at 4 h of infection indicate the capacity of promastigotes to enter macrophages (internalization rate), whereas the numbers obtained after 48 h of culture indicate the capacity of the amastigotes for intracellular multiplication and for infecting new cells (progression rate). Comparisons were made of those parameters in macrophage cultures infected with promastigotes preincubated at 34°C, with and without GM-CSF, and with promastigotes and amastigotes in the presence of affinity-purified anti-GM-CSF antibodies or normal goat immunoglobulin G (IgG) used as control. In vivo studies were conducted by measuring lesion size in BALB/c mice infected in the hind footpads with culture-derived stationary-phase promastigotes and lesion-derived amastigotes. Disease progression was assessed by comparing the sizes of the infected and noninfected footpads.

Measurement of GM-CSF activity. GM-CSF present in the supernatants of infected macrophages was measured by GM-CSF–interleukin-3-dependent cell proliferation, as described previously (21). Briefly, the myelomonocytic cell line FDC-P1 was removed from its maintenance medium, washed three times in Hanks balanced salt solution, and resuspended in RPMI 1640–10% fetal calf serum–10 mM L-glutamine to the desired cell concentration. Then 2×10^4 cells per well were plated in a 96-well round-bottom culture plate and incubated for 48 h at 37°C in a 5% CO_2 atmosphere in the presence of macrophage supernatants (20%, vol/vol). At 18 h before termination, the culture was pulsed with 1 μCi of [^{3}H]thymidine (specific activity, 247.9 GBq/mmol; New England Nuclear, Boston, Mass.) per well, the cells were collected on fiberglass filter paper, and the isotope incorporation was measured by liquid scintillation.

Statistical analysis. Differences between experimental groups were determined by using the two-tailed Student *t* test for unpaired samples.

RESULTS

GM-CSF modulates the infectivity of a population of promastigotes. Resident peritoneal macrophages from BALB/c mice were infected with stationary-phase promastigotes after preincubation for 4 h with and without 100 U of rmGM-CSF per ml at 28, 34, and 37°C. At 4 h after initiation of culture, the internalization rate was assessed by measuring the percentage of infected macrophages and the number of amastigotes per infected cell. As seen in Table 1, treatment of the parasites at 28°C with GM-CSF reduces both parameters of infection. In contrast, preincubation at 34 and 37°C with GM-CSF increases their internalization into macrophages over that of parasites preincubated in the absence of the factor. The progression of infection, assessed by counting the number of amastigotes after 48 h of culture, is affected in the same way: it is reduced by preincubation at 28°C and augmented by preincubation at 34 and 37°C (results not shown).

GM-CSF protects promastigotes from heat-induced death. To clarify the mechanism by which GM-CSF exerts the above-described phenomenon, we measured the effect of the hormone on the thermotolerance of promastigotes. Figure 1 shows the effect on the viability of promastigotes incubated for 4 and 8 h at 37°C, in the absence and in the presence of 100 U of rmGM-CSF per ml, as measured by the conversion of MTT, a substrate for various mitocondrial dehydrogena-

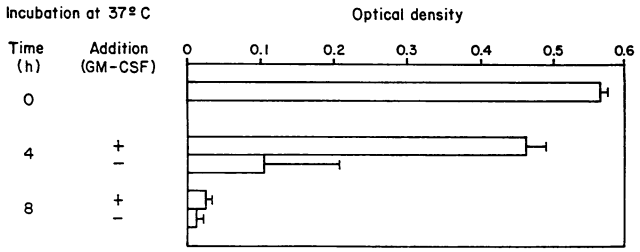

FIG. 1. Effect of heat shock on the survival of *L. amazonensis* promastigotes. MTT activity was measured in triplicate cultures of 10^7 promastigotes after different periods of incubation at 37°C, in the presence and in the absence of 100 U of rmGM-CSF per ml. Bars show the mean values and standard deviations of triplicate determinations from one of four experiments.

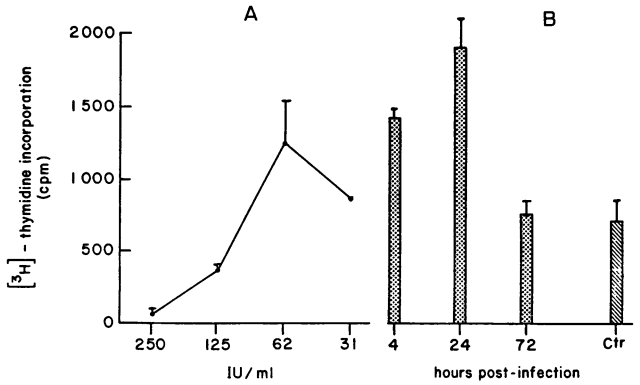

FIG. 2. GM-CSF activity measured by the proliferation of the addicted cell line FDC-P1. (A) Dose-response curve of FDC-P1 cell line proliferation induced by rmGM-CSF. (B) GM-CSF activity present in the supernatants of murine peritoneal macrophages collected at different times postinfection with *L. amazonensis* promastigotes and measured by proliferation of the FDC-P1 cell line. Results represent mean values and standard deviations of triplicate cultures from one of five experiments. The control value (Ctr) represents the activity of a supernatant of noninfected macrophages after 24 h of incubation.

ses (17). The figure shows that cell viability decreases in a time-dependent manner when the cells were incubated in the absence of the recombinant hormone. However, promastigotes are significantly protected by GM-CSF from death for up to 4 h of incubation at 37°C. Furthermore, the thermotolerance-inducing activity of GM-CSF was confirmed by assessing the viability of the heat-shocked parasites by vital-dye exclusion (results not shown).

Anti-GM-CSF antibody inhibits the infectivity of promastigotes but not of amastigotes of *L. amazonensis*. Using a dependent cell line, we showed that macrophage infection at 34°C with *L. amazonensis* induces very early production of GM-CSF. Figure 2B shows the GM-CSF activities in supernatants from infected macrophages after different times of infection, measured by FDC-P1 cell line proliferation, and Fig. 2A shows a dose-response curve of the same cell line to rmGM-CSF. It is clear that as early as 4 h postinfection, large amounts of biologically active GM-CSF can be found in the supernatants of the infected cells. Within this context, an anti-GM-CSF antibody should decrease the infectivity of a population of promastigotes by reducing the number of thermotolerant forms and exert no effect on the infectivity of amastigotes which are already adapted for survival at this temperature. Table 2 shows the results of different experiments in which promastigotes and amastigotes were incubated with macrophages in the presence of 0.5 μg of affinity-purified anti-GM-CSF or normal goat IgG per ml and the rates of infection were measured. It can be seen that the anti-GM-CSF antibody reduces both the internalization (at 4 h) and progression (at 48 h) rates when macrophages are infected with promastigotes and is unable to alter the rate of infection when amastigotes are used to infect the cells. In this last situation, no internalization rate could be measured since the amastigotes, because of their small size, can be easily confused with small cellular debris still present in the early cultures. Similar results were obtained when BALB/c mice were infected with both differentiation forms of the parasite in the presence and in the absence of anti-murine GM-CSF. Figure 3 shows the progression of lesions in mice injected in the footpads with 2×10^7 promastigotes or 2×10^6 amastigotes of *L. amazonensis* together with 5 μg of the same anti-GM-CSF antibody used for the in vitro infections per ml. The anti-GM-CSF antibody is able to slow lesion progression in animals infected with promastigotes (Fig. 3B) but has no such effect on the progression of disease in animals infected with amastigotes (Fig. 3A). In this last situation an increase in lesion size was consistently observed.

DISCUSSION

Several different microorganisms produce and respond to vertebratelike hormones (2, 13, 14). Examples of protozoa that use host-produced cytokines for their own growth and differentiation have also been described (1, 8, 16). Here we describe a situation in which such a direct interaction modulates the course of an infectious disease by interfering with the infectivity of a parasite. We postulate that this type of interaction is part of the natural history of a leishmanial infection.

GM-CSF, in the form of a recombinant molecule, exerts a temperature-dependent effect on the ability of *L. amazonensis* promastigotes to infect macrophages. Incubation of promastigotes with the hormone at 28°C for 4 h preceding infection decreases the infectivity of the parasite, whereas incubation at 34 or 37°C produces the opposite effect (Table 1). In the first situation, fewer parasites are internalized and these are less effective in intramacrophage multiplication when infectivity is compared with that of parasites preincubated in the absence of GM-CSF. In the second situation, however, more promastigotes, with a fully preserved capacity to differentiate into amastigotes and multiply, enter the macrophages, and consequently the overall infectivity of the parasite population is significantly increased. The effect observed at 28°C can be explained by the ability of GM-CSF to induce cell proliferation in the promastigotes (1). Since the infectivity of those parasites is cell cycle dependent (19), we can postulate that the metabolic changes induced by the interaction with the growth factor shift them from a fully infective stage to a low-internalization stage. This hypothesis is still to be confirmed. At 34 and 37°C the survival of the promastigote population is increased by interaction with GM-CSF, and thus more promastigotes are able to infect macrophages. This was observed by comparing promastigote viability, using the MTT assay (Fig. 1), of parasite populations preincubated with and without the hormone, at a high temperature (37°C) for different periods. The mechanism by which GM-CSF exerts its protective effect on promastigotes is yet to be established. It is interesting that the action of GM-CSF as a hematopoietic factor is also to

TABLE 2. Effect of affinity-purified anti-GM-CSF antibody on the infectivity of promastigotes and amastigotes of *L. amazonensis*[a]

Expt no.[b]	Infectivity index for infection with:					
	Promastigotes (4 h p.i.)[c]		Promastigotes (48 h p.i.)[c]		Amastigotes (48 h p.i.)	
	−Anti-GM-CSF	+Anti-GM-CSF	−Anti-GM-CSF	+Anti-GM-CSF	−Anti-GM-CSF	+Anti-GM-CSF
1	11.4	4.2	63.0	29.0	639.0	639.0
2	21.6	9.4	97.0	70.8	185.0	171.0
3	12.6	6.9	142.0	19.1	474.7	546.3
4	46.0	39.0	368.0	139.4		
5	174.0	166.0	498.0	218.0		

[a] Infections were performed with a 5:1 ratio of promastigotes per macrophage and 0.5:1 amastigotes per macrophage.
[b] Experiments 1, 2, and 3 were performed with a strain less infective (H-21) than the one used in experiments 4 and 5 (LV-79).
[c] Experiments performed with and without GM-CSF showed differences of $P < 0.10$.

protect bone marrow precursor cells from death (26). It has already been shown that a particular heat shock protein protects *Saccharomyces cerevisiae* from death at very high temperatures (20). A heat shock protein-dependent thermotolerance mechanism in *Leishmania* spp. is amenable to experimental procedures (27), and its description will be very helpful in understanding the host-parasite interaction. Preliminary results from our laboratory indicate that GM-CSF induces calcium release from promastigotes subjected to heat shock.

Considering that our experimental method for measuring the in vitro infectivity of promastigotes is conducted at 34°C, it is reasonable to expect that the GM-CSF produced in large amounts by the macrophages (Fig. 2B) increases the number of surviving forms within an infecting population of promastigotes via direct interaction with the parasite. Since the indicator cell line used in this assay responds to GM-CSF and interleukin-3 (3), and since no interleukin-3 mRNA can be detected in murine macrophage cultures even after lipopolysaccharide stimulation (21), it becomes clear that the cytokine being measured during infection is really GM-CSF. In this context, the observed effect of an anti-GM-CSF antibody reducing the infectivity rates of promastigotes (Table 2) reinforces the hypothesis that a direct effect of the hormone on the parasite is important for the survival of the infectious form at 34°C. It is interesting that no effect of the antibody was observed in in vitro infections with amastigotes (Table 2). This form of the parasite is naturally adapted to the temperature of the mammalian host and suffers no heat shock during an ongoing infection. Furthermore, the fact that similar results were obtained in an experimental infection in susceptible mice (Fig. 3) indicates that the postulated mechanism also operates in vivo and can be important in determining the outcome of naturally occurring infections.

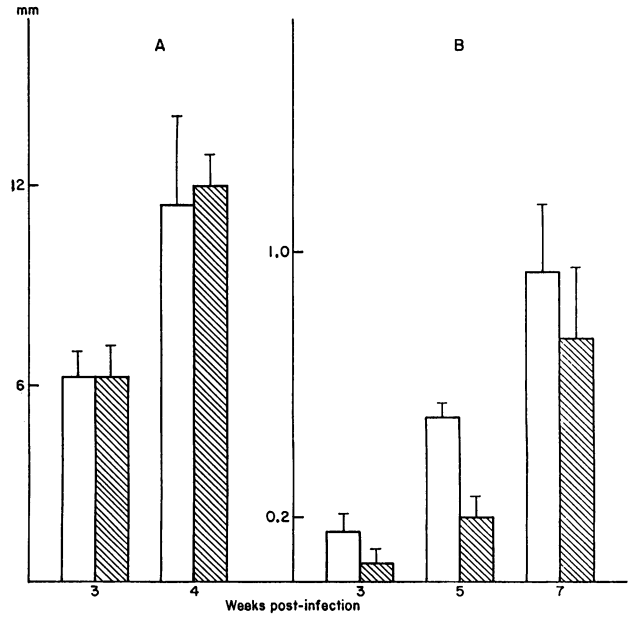

FIG. 3. Lesion size in the footpad of BALB/c mice infected with amastigotes (A) and promastigotes (B) of *L. amazonensis* in the presence of affinity-purified anti-GM-CSF or normal goat IgG. Affinity-purified anti-GM-CSF antibodies (5 μg/ml) (▨) or normal goat IgG (□) were injected at the moment of infection and at 24 and 48 h postinfection in groups of five BALB/c mice. The contralateral footpad, used as a control, received the same doses of anti-GM-CSF or normal goat IgG at the same times. Results are expressed as the means and standard deviations of the differences between the experimental and control footpad of each mouse per group.

The above results allow us to propose a novel mechanism for host-parasite interaction in which growth and differentiation factors produced by the host are used by the microorganism for its own survival. The encounter of promastigotes of *L. amazonensis* with host-produced GM-CSF immediately after the passage of the parasite from the disease-transmitting insect to the skin of the warm-blooded mammal increases the percentage of surviving forms of the infecting population. Considering that most of the transmitting flies, at least as shown with *Phlebotomus papatasi* and *Leishmania major*, egest only 7 to 100 parasites (24) and that fewer than 0.1% of the injected forms can be recovered 24 h after infection (9), an increase in the survival rate of the inoculated promastigote will have a dramatic effect on the fate of the infection. Indeed, we have shown that, in in vitro infection with *L. amazonensis*, macrophages from susceptible BALB/c mice produce significantly larger amounts of GM-CSF than do macrophages from the resistant C57BL/10 strain (20a). One of the most restrictive factors in the present hypothesis, i.e., the encounter of the parasite with the secreted hormone, is postulated to happen in the microenvironment generated by the bite of the transmitting sand fly, where GM-CSF-producing macrophages encounter infective promastigotes. It has been shown that sand fly saliva enhances leishmanial infection (22) by interfering with the ability of gamma interferon to activate macrophages (23). At present we are assessing the ability of sand fly saliva to induce murine macrophages to produce GM-CSF, since this

hormone also inhibits macrophage activation by gamma interferon (6). Our results can explain why different methods of measuring the effect of GM-CSF on the fate of a leishmanial lesion yield contradictory results. GM-CSF enhances the lesion when interacting with promastigotes (5), but it accelerates the cure of the disease by activating macrophage leishmanicidal activity in already established infections (7, 10) or during pretreatment of macrophages (25), and thus the disease is exclusively dependent on the infectivity of amastigotes.

REFERENCES

1. **Charlab, R., C. Blaineau, D. Schechtman, and M. A. Barcinski.** 1990. Granulocyte-macrophage colony stimulating factor is a growth-factor for promastigotes of *Leishmania mexicana amazonensis*. J. Protozool. **37**:352–357.
2. **Csaba, G., and T. Lantos.** 1973. Effect of hormones on Protozoa. Studies on the phagocytic effect of histamine, 3-hydrozytripamine and indoleacetic acid on *Tetrahymena pyriformis*. Cytobiologie **7**:361–365.
3. **Dexter, T. M., J. Garland, D. Scott, E. Scolnick, and D. Metcalf.** 1980. Growth of factor-dependent hemopoietic precursor cell lines. J. Exp. Med. **152**:1036–1047.
4. **Feng, Z. Y., J. Louis, V. Kindler, T. Pedrazzini, F. Eliason, R. Behin, and P. Vassali.** 1988. Aggravation of experimental cutaneous leishmaniasis in mice by administration of interleukin 3. Eur. J. Immunol. **18**:1245–1251.
5. **Greil, E., B. Bodendorfer, M. Rollinghoff, and W. Solbach.** 1988. Application of recombinant granulocyte-macrophage colony-stimulating factor has a detrimental effect in experimental murine leishmaniasis. Eur. J. Immunol. **18**:1527–1533.
6. **Hancock, W. W., M. E. Pleau, and L. Kobzik.** 1988. Recombinant granulocyte-macrophage colony-stimulating factor downregulates expression of IL-2 receptor on human mononuclear phagocytes by induction of prostaglandine. J. Immunol. **140**: 3021–3025.
7. **Handman, E., and A. W. Burgess.** 1979. Stimulation by granulocyte-macrophage colony-stimulating factor of *L. tropica* killing by macrophages. J. Immunol. **122**:1134–1137.
8. **Hide, G., A. Gray, C. M. Harrison, and A. Tait.** 1989. Identification of an epidermal growth factor receptor homologue in trypanosomes. Mol. Biochem. Parasitol. **36**:51–60.
9. **Hill, J. O., R. J. North, and F. M. Collins.** 1983. Advantages of measuring changes in the number of viable parasites in murine models of experimental cutaneous leishmaniasis. Infect. Immun. **39**:1087–1094.
10. **Ho, J. L., S. G. Reed, E. A. Wick, and M. Giordano.** 1990. Granulocyte-macrophage colony-stimulating factors activate intramacrophagic killing of *Leishmania mexicana amazonensis*. J. Infect. Dis. **162**:224–230.
11. **Kiderlen, A. F., and P. M. Kaye.** 1990. A modified colorimetric assay of macrophage activation for intracellular cytotoxicity against *Leishmania* parasites. J. Immunol. Methods **127**:11–18.
12. **Lelchuk, R., R. Graveley, and F. Y. Liew.** 1988. Susceptibility to murine cutaneous leishmaniasis correlates with the capacity to generate interleukin-3 in response to *Leishmania* antigen *in vitro*. Cell. Immunol. **111**:66–76.
13. **Le Roith, D., A. S. Liotta, J. Roth, J. Shiloach, M. E. Lewis, C. B. Pert, and D. T. Krieger.** 1982. Corticotropin and endorphin like materials are native to unicellular organisms. Proc. Natl. Acad. Sci. USA **79**:2086–2090.
14. **Le Roith, D., J. Shiloach, J. Roth, and M. A. Lesniak.** 1980. Evolutionary origins of vertebrate hormone substances similar to mammalian insulins are native to unicellular organisms. Proc. Natl. Acad. Sci. USA **77**:6184–6188.
15. **Mauel, J.** 1990. Macrophage-parasite interactions in *Leishmania* infections. J. Leukocyte Biol. **47**:187–193.
16. **Mazingue, C., F. Cottrez-Detoeuf, J. Louis, M. Kweider, C. Aurialt, and A. Capron.** 1989. *In vitro* and *in vivo* effects of interleukin-2 on the protozoan parasite leishmania. J. Immunol. **19**:487–491.
17. **Mosmann, T.** 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods **65**:55–63.
18. **Sacks, D. L., S. Hieny, and A. Sher.** 1985. Identification of cell surface carbohydrate and antigenic changes between noninfective and infective developmental stages of *Leishmania major* promastigotes. J. Immunol. **135**:564–569.
19. **Sacks, D. L., and P. V. Perkins.** 1984. Identification of an infective stage of *Leishmania* promastigotes. Science **223**:1417–1419.
20. **Sanchez, Y., and S. L. Lindquist.** 1990. HSP 104 required for induced thermotolerance. Science **248**:1112–1115.

20a. **Soares, L. R. B., and M. A. Barcinski.** 1992. J. Leukocyte Biol. **51**:220–224.

21. **Thorens, B., J. Mermod, and P. Vassali.** 1987. Phagocytosis and inflammatory stimuli induce GM-CSF mRNA in macrophages through posttranscriptional regulation. Cell **48**:671–679.
22. **Titus, R. G., and J. M. C. Ribeiro.** 1988. Salivary gland lysates from the sand fly *Lutzomyia longipalpis* enhance *Leishmania* infectivity. Science **239**:1306–1308.
23. **Titus, R. G., and J. M. C. Ribeiro.** 1990. The role of vector saliva in transmission of arthropod-borne disease. Parasitol. Today **6**:157–160.
24. **Warburg, A., and Y. Schlein.** 1986. The effect of post-bloodmeal nutrition of *Phlebotomus papatasi* on the transmission of *Leishmania major*. Am. J. Trop. Med. Hyg. **35**:926–930.
25. **Weiser, W. Y., A. Van Niel, C. Clark, J. R. David, and H. G. Remold.** 1987. Recombinant human granulocyte/macrophage colony-stimulating factor activates intracellular killing of *Leishmania donovani* by human monocyte-derived macrophages. J. Exp. Med. **166**:1436–1446.
26. **Williams, G. T., C. A. Smith, E. Spooncer, T. M. Dexter, and D. R. Taylor.** 1990. Haemopoietic colony stimulating factors promote cell survival by suppressing apoptosis. Nature (London) **343**:76–79.
27. **Young, D., R. Lathigra, and A. Mehlert.** 1989. Stress-induced proteins as antigens in infectious diseases, p. 275–285. *In* Stress-induced proteins. Alan R. Liss, Inc., New York.