RECOMBINANT GRANULOCYTE/MACROPHAGE COLONY-STIMULATING FACTOR ACTIVATES MACROPHAGES TO INHIBIT *TRYPANOSOMA CRUZI* AND RELEASE HYDROGEN PEROXIDE

Comparison with Interferon γ

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Trypanosoma cruzi, the etiologic agent of Chagas' disease, replicates in the cytoplasm of mononuclear phagocytes. In vitro, factors released by antigen- or mitogen-stimulated T lymphocytes can activate macrophages to inhibit the growth of this protozoan (1, 2). Interferon γ (IFN-γ) appears to be one of the principal lymphokines that can activate macrophages for resistance to a wide variety of intracellular microbial pathogens (reviewed in 3), including *T. cruzi* (4, 5). However, there are additional macrophage-activation factors (6–10), some of which may have a narrower spectrum of activity (7, 8). Defined cytokines with ability to enhance macrophage antimicrobial activity include recombinant tumor necrosis factor α (rHu-TNF-α) (tested with *T. cruzi* [8]) and partially purified murine granulocyte/macrophage colony-stimulating factor (Mu-GM-CSF)[1] (tested with leishmania [9]). In addition, a two- to threefold stimulation of macrophage respiratory burst capacity was reported with partially purified macrophage colony-stimulating factor (M-CSF) (10). Studies with recombinant cytokines are needed to confirm macrophage activation by CSFs, characterize their spectrum of activity, establish their potency, and explore their mechanisms of action.

The cloning and expression (11), and purification of murine GM-CSF now permit the study of its macrophage-activating potential using a well-characterized population of tissue macrophages, those from the mouse peritoneal cavity. Such studies are reported below, along with comparative experiments using rMu-IFN-γ and experiments using human monocytes and macrophages stimulated with

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[1] *Abbreviations used in this paper:* EC_{50}, 50% effective concentration; GM-CSF, granulocyte/macrophage colony-stimulating factor; Hu-, human; M-CSF, macrophage CSF; Mu-, murine; PC, peritoneal cells.

rHu-GM-CSF and Hu-IFN-γ. In addition, we have compared the potency of rMu-GM-CSF, rHu-M-CSF, rHu-TNF-α, and rMu-IFN-γ for enhancement of mouse peritoneal macrophage respiratory burst capacity, a close correlate of their antitrypanosome (12), antitoxoplasma (13, 14), and antileishmania (14, 15) activity.

Materials and Methods

Mice. For tests with *T. cruzi*, female BALB/cByJ mice were purchased from The Jackson Laboratories (Bar Harbor, ME) and used at 8–12 wk of age. For tests of H_2O_2-releasing capacity, specific pathogen-free female BALB/c mice were purchased from Taconic Farms (Germantown, NY) and housed under barrier conditions with autoclaved food, water, and bedding.

Parasite. The Tulahuen isolate of *T. cruzi* (MHOM/CH/00/Tulahuen) was cloned by twice-repeated microdrop selection. The clone used in these studies was designated as MHOM/CH/00/Tulahuen C2. Trypomastigotes were produced by inoculating bloodstream parasites from an infected mouse onto monolayers of the J774A.1 mouse macrophage cell line (American Type Culture Collection, Rockville, MD) grown in RPMI 1640 medium (M.A. Bioproducts, Walkersville, MD) with 10% FCS (M.A. Bioproducts) and harvesting extracellular trypomastigotes from the cultures 5–6 d later, as described (16). Parasites were separated from contaminating host cells by centrifugation on lymphocyte separation medium (Litton Bionetics, Kensington, MD) and frozen at −80°C until use. These parasites produced acute, fatal infections in BALB/c mice (LD_{50} ~25 trypomastigotes).

Microbicidal Assays. Mouse resident peritoneal cells (PC) were collected by peritoneal lavage and were plated on 12-mm-diam glass coverslips ($2.8–3 \times 10^5$ macrophages/coverslip) or placed in polypropylene tubes (Falcon Labware, Oxnard, CA) at 5×10^5 macrophages/tube in 0.5 ml RPMI/5% FCS. Culture media and FCS contained <0.01 ng/ml bacterial endotoxin as determined by the Limulus amoebocyte lysate assay (M.A. Bioproducts). Cells applied to coverslips were allowed to adhere for 2 h, and then washed vigorously to remove nonadherent cells. Infection of PC in adherent or suspension cultures was carried out at a 1:1 parasite/macrophage ratio. After 2 h of infection, suspension cultures were centrifuged (150 *g*, 10 min) to remove extracellular trypanosomes, and resuspended in fresh RPMI/10% FCS. Adherent cell cultures were rinsed thoroughly with RPMI and cultured in RPMI/10% FCS with or without cytokine, for 5–6 d at 37°C. At the end of the incubation period, aliquots of the suspension cultures were removed for total cell and parasite counts. Smears were prepared by cytocentrifugation, fixed with methanol, and stained with Giemsa for determination of intracellular parasite numbers. Adherent cell cultures were stained with Giemsa for counting intracellular parasites.

Human peripheral blood monocytes were isolated as previously described (17). Ficoll-Hypaque (Isolymph; Gallard-Schlesinger, Inc., Carle Place, NY) purified peripheral blood leukocytes were fractionated by Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) continuous gradient centrifugation. The monocyte-enriched fraction was allowed to adhere to 12-mm-diam glass coverslips (10^6 cells/coverslip) for 1.5 h in RPMI/5% FCS. Nonadherent cells were then removed with three washes. The adherent cells were cultured in RPMI/5% FCS (1 ml/well) in 24-well plates (Corning Glass Works, Corning, NY) for 2 h. Macrophages were derived by incubating the cells for 7 d without added lymphokine. Infection of monocytes or macrophages was carried out at a 1:1 parasite/cell ratio.

Microbistatic activity was calculated as described (18) by the formulae: $100 \times$ [(parasites per 100 infected control cells) − (parasites per 100 infected treated cells)]/(parasites per 100 infected control cells); and $100 \times$ [(percent infected control cells) − (percent infected treated cells)]/(percent infected control cells).

Cytokines. Mu-GM-CSF was cloned from an LBRM-33 mC9 cDNA library and expressed in yeast. Recombinant protein was purified to homogeneity from yeast supernatant using two cycles of reversed-phase HPLC as previously described (19). The specific activity of purified rMuGM-CSF is 5×10^7 U/μg, as measured in the mouse bone marrow

proliferation assay (20). Hu-GM-CSF was cloned, expressed, and purified to homogeneity as previously described (21). The specific activity of rHu-GM-CSF is 5×10^5 CFU-C/mg, as measured in the human bone marrow colony assay (18). rMu-IFN-γ (17.4×10^6 U per 1.4 mg protein) was provided by Genentech, Inc., South San Francisco, CA, as protein purified to homogeneity from *Escherichia coli* (lot 3209-33). Natural Hu-IFN-γ (1.5×10^6 U/mg) was purchased from Meloy Laboratories (Springfield, VA) and was determined to be free of bacterial endotoxin as above. rHu-TNF-α, expressed in *E. coli* and purified to homogeneity, was the gift of Genentech, Inc., and had a specific activity of 7.6×10^7 U/mg protein in a cytotoxicity assay against actinomycin D–treated L929 cells (22). rHu-M-CSF, expressed in Chinese hamster ovary cells and purified to homogeneity, was the gift of Genetics Institute, Inc., Cambridge, MA, and had a specific activity of $2.5–5 \times 10^5$ U/mg protein in a mouse bone marrow assay (23).

H_2O_2-releasing Capacity and Measurement of Adherent Cell Number and Protein. Using the materials and methods previously described (24), resident mouse peritoneal cells were plated in triplicate in 96-well plastic tissue culture trays at 2×10^5 cells/well, washed after 2 h to remove nonadherent cells, and either assayed immediately or exposed to cytokines for 1, 2, or 3 d. At each time, one set of washed plates (see below) was used to measure H_2O_2 release in response to phorbol myristate acetate (PMA), by following the horseradish peroxidase–dependent oxidation of fluorescent scopoletin to a nonfluorescent product at intervals over 2 h in a plate-reading fluorometer. The protein content of each well was then determined with a modification of the method of Lowry et al. (25). Both methods have been described in detail (26). Another set of plates was washed in the same way and used to determine the number of adherent cells by lysing the cells and counting the nuclei in a hemocytometer, as described (27). Tightly adherent cells are defined as those that remained after the plates were flicked hard enough to remove all visible fluid, immersed by saline, and flicked again, this procedure being repeated twice more (26).

Statistics. Means and SEM or SD are shown for the number of experiments indicated. p values are based on Student's t test.

Results

Inhibition of T. cruzi in Mouse Peritoneal Macrophages by rMu-GM-CSF and rMu-IFN-γ. The capacity of rMu-GM-CSF to induce antimicrobial activity in mouse resident peritoneal macrophages was evaluated by adding the cytokine either before or after infection with *T. cruzi*.

For the first set of experiments, glass-adherent macrophages were incubated with 10 or 100 ng rMu-GM-CSF/ml for 24–72 h before infection with *T. cruzi*. Intracellular parasite numbers were determined at 2 or 4 h after infection, and again at 96 h after infection. As shown in Table I, pretreatment of PC with rMu-GM-CSF did not consistently affect parasite uptake, as determined by parasite numbers at 2 or 4 h after infection. However, when parasite numbers were determined 96 h later, significant ($p < 0.01$) inhibition of the increase in percent macrophages infected and of the number of amastigotes per 100 infected macrophages was noted in macrophages pretreated with rMu-GM-CSF. Inhibition was not significantly different whether preincubation proceeded for 24, 48, or 72 h before infection, or whether 10 or 100 ng rMu-GM-CSF/ml was used. However, the overall antitrypanosomal effect was microbistatic rather than -cidal, in that the number of trypomastigotes per 100 macrophages increased even in rMu-GM-CSF–treated cells.

In the second set of experiments, peritoneal macrophages were infected with *T. cruzi* and washed before addition of different concentrations of rMu-GM-CSF. 5 d later, the numbers of intracellular parasites were determined (Fig. 1).

TABLE I

Effects of Preincubation of Mouse Peritoneal Macrophages with rMu-GM-CSF on Their Ability to Inhibit Intracellular Replication of T. cruzi

Exp.	rMu-GM-CSF*	Time after infection‡	Cells infected (mean ± SD)	Δ	Inhibition§	Amastigotes per 100 infected macrophages	Δ	Inhibition§
	ng/ml	*h*	%		%			%
A	0	2	5.4 ± 4.0	9.1	—	5.9 ± 4.8	78.8	—
	0	96	14.5 ± 2.1			84.7 ± 25.8		
	10	2	4.5 ± 1.1	0.7	92	4.5 ± 1.1	22.0	72
	10	96	5.2 ± 2.4			26.5 ± 2.1		
	100	2	10.2 ± 3.8	−2.3	100	10.9 ± 2.4	15.4	80
	100	96	7.9 ± 8.6			26.3 ± 8.9		
B	0	2	5.1 ± 3.0	2.6	—	5.1 ± 3.0	58.0	—
	0	96	7.8 ± 1.8			63.8 ± 4.9		
	10	2	4.8 ± 1.5	0.4	85	9.1 ± 2.7	16.1	73
	10	96	5.2 ± 2.6			25.2 ± 16.7		
	100	2	3.9 ± 4.0	−0.9	100	3.4 ± 3.0	13.0	78
	100	96	3.0 ± 2.2			16.4 ± 14.0		
C	0	4	14.2 ± 4.0	27.0	—	23.2 ± 11.0	137.8	—
	0	96	41.0 ± 22.1			161.0 ± 110.1		
	10	4	14.6 ± 3.9	−0.6	100	21.0 ± 7.0	41.2	70
	10	96	14.0 ± 0.0			62.2 ± 7.1		

* Resident peritoneal macrophages were incubated 24 (*A*), 48 (*B*), or 72 h (*C*) in different concentrations of rMu-GM-CSF before infection with *T. cruzi* trypomastigotes.

‡ Numbers of intracellular parasites were determined at 2 or 4 h, and at 96 h after infection.

§ Percent inhibition of *T. cruzi* in macrophages preincubated with rMu-GM-CSF compared with macrophages preincubated in medium alone.

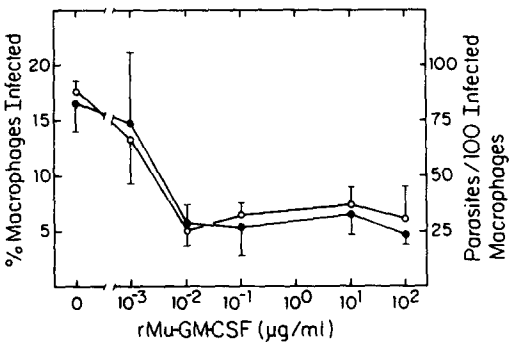

FIGURE 1. Inhibition of *T. cruzi* in mouse peritoneal macrophages by treatment with rMu-GM-CSF after infection in vitro. Macrophages adherent to glass coverslips were infected with *T. cruzi* for 2 h before addition of the indicated concentration of rMu-GM-CSF. After 5 d, the coverslips were stained with Giemsa and numbers of intracellular parasites were determined. Results are means ± SD for triplicate cultures from each of two experiments. Significant ($p < 0.05$) inhibition, as compared with cultures receiving no rMu-GM-CSF, was achieved with doses of 10^{-2}–10^{2} μg/ml. (●) Percent macrophages infected; (○) parasites per 100 infected macrophages.

Treatment with rMu-GM-CSF at 10 ng/ml to 100 μg/ml resulted in marked microbistatic activity, expressed as reduction in the number of intracellular parasites per 100 infected cells, and in the percentage of cells infected compared with untreated cultures on day 5. Direct enumeration of macrophages on the glass coverslips was determined as described (27). No net increase of mouse resident PC was obtained with any of the concentrations of cytokines tested (data

TABLE II

Microbistatic Activity of Mouse Peritoneal Cells Stimulated with rMu-GM-CSF

rMu-GM-CSF	Microbistatic activity	
	Adherent cultures	Suspension cultures
ng/ml	%	
0	0	0
1	30.4	9.5
10	83.5	71.4
10^2	64.7	42.8

Mouse PC were cultured as adherent cells on glass coverslips or in suspension in polypropylene culture tubes for 2 h before infection with *T. cruzi* and subsequent addition of the indicated concentrations rMu-GM-CSF. After incubation for 5 d, the cells were stained and intracellular parasites counted.

not shown). Thus the observed decrease in percentage of infected cells after cytokine treatment was not due to an increase in macrophage number.

To test the induction of microbistatic activity using mouse PC in suspension, PC were infected in polypropylene culture tubes, washed and incubated in these tubes for 5 d. The cells were then counted and slides prepared by cytocentrifugation. In Table II, the results of suspension cultures are compared with results obtained from adherent cell cultures. The suspension cultures inhibited parasite replication to a similar extent as adherent cell cultures, without a net increase in macrophage numbers.

For comparison with microbistatic activity of rMu-GM-CSF, additional cultures of adherent mouse PC were infected with trypomastigotes, washed 2 h after infection to remove free parasites, and incubated in different amounts of rMu-IFN-γ. Intracellular amastigotes were counted 5–6 d later. rMu-IFN-γ was extremely effective at inhibiting intracellular replication of *T. cruzi* in mouse peritoneal macrophages, at doses as low as 0.1 ng/ml (Table III). In all experiments, inhibition of the number of amastigotes per 100 cells was $\geq$95% at 1 ng/ml. Neither rMu-GM-CSF nor rMu-IFN-γ was toxic for *T. cruzi* trypomastigotes. After a 24 h incubation in the presence of different concentrations of rMu-IFN-γ or rMu-GM-CSF, there was no decrease in parasite number (Table IV) or motility (not shown).

H_2O_2-releasing Capacity and Measurement of Adherent Cell Number and Protein. These experiments were performed with resident macrophages from specific pathogen–free mice. As previously reported (28), in the absence of exogenous cytokines, the number of such cells tightly adherent to the culture surface declined by 79% over 3 d of culture. Each of the cytokines tested (rMu-GM-CSF, rHu-M-CSF, rHu-TNF-α, and rMu-IFN-γ) retarded this decline. Most effective was rHu-M-CSF, which resulted in as many or more cells remaining tightly adherent on day 3 as were adherent on day 0 (Fig. 2*A*). In contrast, even in the absence of added cytokines, the adherent cells increased in protein content over the same period. Each of the four cytokines augmented this increase, rMu-GM-CSF being the most active (Fig. 2*B*). Finally, each of the cytokines markedly

TABLE III

Inhibition of T. cruzi in Mouse Peritoneal Macrophages Treated with rMu-IFN-γ after Infection

Exp.	rMu-IFN-γ	Macrophages infected (mean ± SD)	Inhibition	Amastigotes per 100 infected macrophages (mean ± SD)	Inhibition
	ng/ml	%	%		%
1	0	11.7 ± 0.1	—	86.5 ± 20.8	—
	0.01	11.6 ± 0.5	0	89.4 ± 13.9	0
	1.0	0	100	0	100
2	0	7.4 ± 0.7	—	64.9 ± 32.9	—
	0.01	12.8 ± 6.0	0	62.2 ± 37.4	4
	0.1	4.2 ± 5.5	43	14.5 ± 17.7	78
	1.0	0.3 ± 0.4	96	0.7 ± 0.9	99
3	0	23.1 ± 12.2	—	473.8 ± 125.6	—
	0.1	2.0 ± 0	91	10.1 ± 1.1	98
	1.0	2.5 ± 2.0	89	8.0 ± 7.1	98

Unelicited mouse peritoneal macrophages were adhered to glass coverslips, infected with *T. cruzi*, washed, and incubated for 5 d with or without rMu-IFN-γ.

TABLE IV

rMu-GM-CSF and rMu-IFN-γ Are Not Toxic for T. cruzi Trypomastigotes

	rMu-GM-CSF or rMu-IFN-γ	Trypomastigotes (mean ± SD)
	ng/ml	× 10^4
Mu-GM-CSF	0	8.1 ± 2.8
	1	9.6 ± 3.1
	10	8.4 ± 1.2
	100	8.0 ± 3.4
Mu-IFN-γ	0.05	10.8 ± 3.2
	5.0	10.5 ± 3.8
	500	10.1 ± 3.5

T. cruzi trypomastigotes (1.5×10^5 motile parasites in 1 ml) were incubated (37°C) with or without cytokine. Numbers of motile parasites were determined after 24 h of incubation. Results are from triplicate cultures of two experiments.

augmented the ability of the macrophages to release H_2O_2, whether the results were expressed per milligram of adherent cell protein (Fig. 2*C*) or per 10^6 macrophages (Fig. 2*D*). Peak effects were seen by 1 d with the CSFs, but not until 2 d with rMu-IFN-γ or rHu-TNF-α. On the other hand, the magnitude of the increase in H_2O_2-releasing capacity was far greater with rMu-IFN-γ (44-fold greater on day 2 than for cells incubated without exogenous cytokines, mean of four consecutive experiments) and rHu-TNF-α (51-fold over control, two exper-

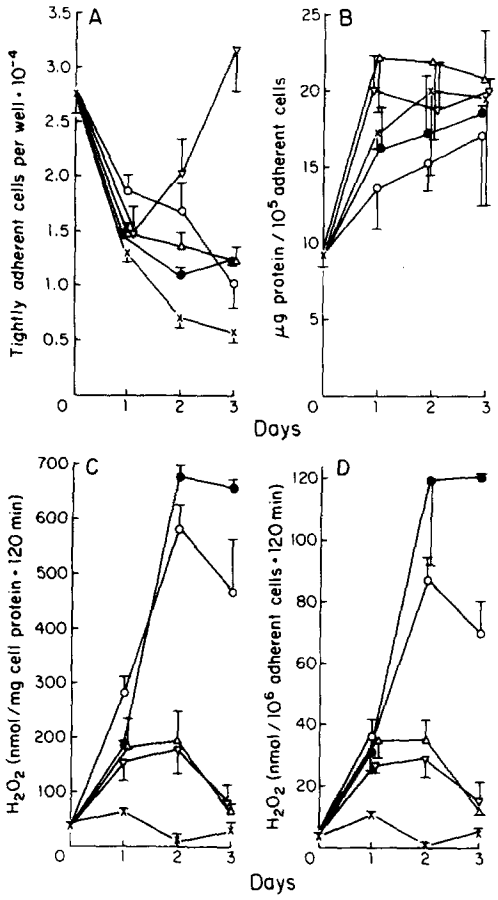

FIGURE 2. Comparison of rMu-GM-CSF (△), rHu-M-CSF (▽), rMu-IFN-γ (○), and rHu-TNF-α (●) with medium control (×) for ability to augment: (*A*) firm adherence of macrophages in monolayer culture, (*B*) protein content per adherent macrophage, and (*C* and *D*) H_2O_2-releasing capacity expressed (*C*) per milligram of adherent cell protein or (*D*) per 10^6 cells. Peritoneal macrophages from untreated, specific pathogen–free mice were cultured in triplicate in 96-well plates in the indicated cytokines or medium alone for 2 h (0 d) or for 1, 2, or 3 d before the plates were vigorously washed. H_2O_2 release was determined in response to PMA and protein was measured in the same wells. Matched wells were used for counting nuclei after lysing the cells. Results are means ± SEM for four consecutive experiments (two for rHu-TNF-α) using 10 or 100 ng/ml rMu-GM-CSF, 100 ng/ml rHu-M-CSF, 1.9 ng/ml rMu-IFN-γ, and 1.9 ng/ml rHu-TNF-α.

iments) than for rMu-GM-CSF (15-fold over control, four experiments) or rM-CSF (14-fold over control, four experiments).

The cytokines also differed markedly in the doses required (Fig. 3). rHu-M-CSF was least potent, requiring 100 ng/ml for optimal enhancement of H_2O_2-releasing capacity, compared with 10 ng/ml for rMu-GM-CSF. The 50% effective concentration (EC_{50}) for rMu-IFN-γ was 20–280 times lower than for the CSFs. The concentrations of cytokines required for enhancement of H_2O_2 releasing capacity were comparable to those required for induction of trypanostatic activity. Combinations of rMu-GM-CSF or rHu-M-CSF with rMu-IFN-γ did not result in synergistic increases in H_2O_2 release per milligram of cell protein. However, such combinations did maximize the total H_2O_2-production capacity per culture, by virtue of greater retention of adherent cells and greater increase in their size (not shown).

In additional experiments (not shown), omission of horseradish peroxidase eliminated the observed loss of fluorescence of scopoletin, confirming that the measurements with CSF-treated cells reflected production of H_2O_2 rather than nonspecific degradation of the fluorophore. In addition, rMu-GM-CSF–treated cells displayed an enhanced respiratory burst when challenged with live, unop-

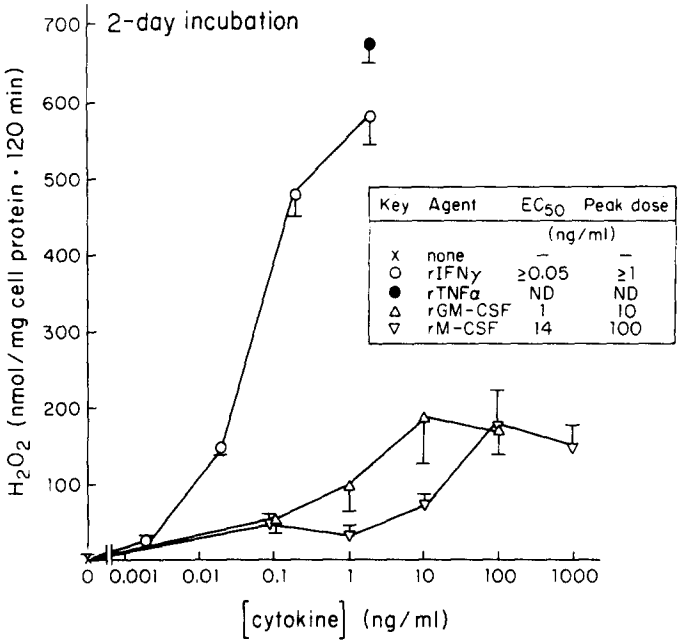

FIGURE 3. Dose dependence of enhancement of H_2O_2-releasing capacity of resident mouse peritoneal macrophages by the cytokines listed in the key. Dose-response curves were tested on each day of culture in the same experiments described in Fig. 2. Results shown above are the means ± SEM for day 2 in each of those experiments. Results for the other days were similar, except that lower doses of rMu-IFN-γ were relatively more effective on day 1 than day 2.

sonized trypomastigotes in place of PMA. No H_2O_2 release from the trypomastigotes themselves was detected under the same conditions. Finally, when neither trypomastigotes nor PMA was added, no H_2O_2 could be detected from untreated macrophages or macrophages treated with any of the cytokines.

Effects of rHu-GM-CSF and Hu-IFN-γ on the Replication of T. cruzi in Human Monocytes and Macrophages. We next tested the inhibition of intracellular replication of *T. cruzi* in human peripheral blood monocytes and macrophages in response to rHu-GM-CSF or partially purified human IFN-γ. Adherent monocytes were cultured for 1 h before infection with *T. cruzi.* The infected monolayers were washed after 2 h and then recultured in the presence or absence of lymphokine for an additional 6 d. Both rHu-GM-CSF and Hu-IFN-γ inhibited the intracellular replication of *T. cruzi,* as shown in Table V. Results were similar if the monocytes were allowed to differentiate into macrophages for 7 d before infection (Table V).

Discussion

These results establish that rMu-GM-CSF can activate macrophages by the dual criteria of enhanced antimicrobial activity and oxidative metabolism although less effectively than rMu-IFN-γ. However, rHu-GM-CSF stimulated antimicrobial activity in human monocytes and macrophages to a degree similar to that observed with IFN-γ. rHu-M-CSF and rHu-TNF-α (24, 29) also enhanced

TABLE V

Inhibition of Intracellular T. cruzi in Human Monocytes and Macrophages by rHu-GM-CSF and IFN-γ

Cells	Cells cultured with:	Parasites per 100 infected monocytes or macrophages (mean ± SD)	Microbistatic activity
			%
Monocytes (day 1–6)	Medium	2,240 ± 470	—
	rHu-GM-CSF (10 ng/ml)	402 ± 122	82
	IFN-γ (1,000 U/ml)	704 ± 609	69
Macrophages (day 7–13)	Medium	290 ± 111	—
	rHu-GM-CSF (10 ng/ml)	85 ± 20	71
	IFN-γ (1,000 U/ml)	73 ± 89	75

Human peripheral blood monocytes and macrophages were prepared as described in Materials and Methods, infected with *T. cruzi* trypomastigotes, and cultured with predetermined optimal doses of cytokine. Numbers of intracellular parasites were determined 5 d after infection. Values are from six individual donors. Results obtained using cytokine differ ($p < 0.05$) from those for the controls.

macrophage H_2O_2-secretory capacity; their antimicrobial effects were not tested. These findings contribute to a growing body of evidence that CSFs, originally defined as growth factors for progenitor cells from marrow, also exert marked effects on the in vitro survival and effector functions of their progeny from blood (9, 10, 18, 30–33).

The contribution of CSFs to macrophage activation in vivo is unknown. GM-CSF, which can be produced in several hours in response to bacterial lipopolysaccharide (34), induced a maximal increase in macrophage H_2O_2-releasing capacity by the first time tested (~18 h), in contrast to IFN-γ, a product of stimulated T cells, which required ~44 h for peak effects. GM-CSF is a product not only of T cells, but also of endothelial cells (35), fibroblasts (35, 36), and macrophages themselves (34), all of which are widely distributed in normal tissues. Thus, after microbial invasion, macrophage activation mediated by GM-CSF can perhaps be attained more quickly than that obtained with IFN-γ, which depends on recruitment and clonal expansion of antigen-specific T cells in an infective focus. Such speculations must be tempered by the fact that the tempo of events in vitro may not accurately reflect that in vivo, where, for example, rMu-IFN-γ fully activates mouse peritoneal macrophages within 18 h (14).

Neither rMu-GM-CSF nor rMu-IFN-γ induced clear-cut trypanocidal activity in these experiments. Rather, they induced macrophages to exert trypanostasis, which was nearly complete in the case of rMu-IFN-γ. The failure of cytokine-treated macrophages to eradicate the parasite in vitro does not preclude their critical participation in effective host defense in vivo. Indeed, mice treated with rMu-IFN-γ showed long-term survival from otherwise rapidly lethal *T. cruzi* infection (S. Reed, manuscript submitted for publication). Likewise, it is not clear that the requirement for relatively high concentrations of CSFs for activation in vitro precludes a physiologic role for these agents as macrophage-activating

factors in vivo. Finally, the role of the respiratory burst in the inhibition of growth of *T. cruzi* has not been established. However, it is possible that the greater efficacy of rMu-IFN-γ–treated macrophages compared with CSF-treated cells can be attributed in part to their relatively greater capacity for a respiratory burst.

Differences in cytokine preparations, the strain of *T. cruzi*, or assay methods may explain the more marked effects observed here than in another study with rMu-IFN-γ (5). Previous experiments did not reveal enhanced H_2O_2-releasing capacity after exposure of human macrophages to partially purified preparations of natural GM-CSF or of M-CSF (37). In retrospect, the concentration of M-CSF used in the earlier work was almost 100-fold lower than would be expected to be effective in light of the present experiments. Likewise, there are contrasting reports on the failure of crude natural GM-CSF (38) versus the efficacy of pure recombinant GM-CSF (39) to activate macrophages to inhibit leishmania. These experiences emphasize the advantage of using pure recombinant materials for analyzing the effects of cytokines individually, and the difficulty of predicting the outcome in situ, where cytokines are likely to be present in complex mixtures.

Summary

Recombinant granulocyte/macrophage colony-stimulating factors (rGM-CSF) of mouse and human origins activated macrophages of the homologous species to inhibit the replication of the protozoan parasite *T. cruzi*. Activation could be induced with 10–100 ng/ml of rMu-GM-CSF, whether it was added before or after uptake of the parasite, in either adherent or suspension cultures. However, the degree of inhibition of parasite replication after exposure to rMu-GM-CSF was not as great as after treatment with rMu-IFN-γ, and much more rMu-GM-CSF than rMu-IFN-γ was required to achieve an equivalent antimicrobial effect. These results were mirrored by effects of the cytokines on enhancement of H_2O_2-releasing capacity in resident mouse peritoneal macrophages. In the latter tests, rMu-IFN-γ and rHu-TNF-α afforded a 44–51-fold enhancement over the untreated control, with a 50% effective concentration (EC_{50}) for rMu-IFN-γ of ~0.05 ng/ml. Using rMu-GM-CSF or rM-CSF, enhancement of H_2O_2-releasing capacity was 14–15-fold over control, with EC_{50}s of 1 and 14 ng/ml, respectively. However, peak enhancement of macrophage H_2O_2-releasing capacity was seen at least 24 h earlier with rMu-GM-CSF or rHu-M-CSF than with r-Mu-IFN-γ or rHu-TNF-α. Thus, rMu-GM-CSF and rHu-GM-CSF displayed clear-cut macrophage-activating activity in vitro, but rMu-GM-CSF was less potent and less effective than rMu-IFN-γ in the tests used.

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