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Gamma Interferon Activates Human Macrophages to Become Tumoricidal and Leishmanicidal but Enhances Replication of Macrophage-Associated Mycobacteria

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Recombinant human gamma interferon (rIFN-γ) was examined for its ability to activate human peripheral blood monocyte-derived macrophages to kill tumor cells and to affect the replication of two phylogenetically distinct intracellular pathogens, *Mycobacterium tuberculosis* and *Leishmania donovani*. Macrophages preincubated overnight with doses of rIFN-γ from 5 to 500 U/ml killed [^{3}H]thymidine-labeled mouse L929 tumor targets, as measured by the release of [^{3}H]thymidine into the supernatant after 48 h. Counts of macrophages initially infected with leishmania promastigotes showed that rIFN-γ-pretreated macrophages could both inhibit the replication of and kill the resulting intramacrophage amastigotes over a 7-day period. However, rIFN-γ pretreatment of macrophages actually enhanced mycobacterial replication over a 5- to 7-day period, as assessed by (i) counting acid-fast bacilli or (ii) lysing macrophages to release bacteria and determining the numbers of viable units. Mycobacterial growth was not affected by rIFN-γ in the absence of macrophages. rIFN-γ pretreatment had opposite effects on the uptake of mycobacteria and leishmania. As many as 80% fewer activated macrophages ingested mycobacteria compared with controls, whereas 50% more activated macrophages were infected with leishmania. These results suggest that rIFN-γ may interfere with the immune destruction of intracellular tubercle bacilli and that the mechanisms of immunity against mycobacteria and leishmania may differ.

Experimental evidence indicates that macrophages can be activated by products of sensitized T cells (lymphokines) to become tumoricidal (macrophage-activating factor [MAF]) (4, 19, 33) or to inhibit or kill intramacrophage pathogens such as mycobacteria (11, 38, 41), listeria (9), legionella (14), rickettsia (24), chlamydia (7, 31), leishmania (22, 25), toxoplasma (27), trypanosoma (28), histoplasma (40), and coccidiodes (2). This ability to be activated plays a role in the pathogenesis of infectious disease and may have a role in neoplasia.

There is some confusion whether MAF is similar to lymphokines that activate macrophages to become microbicidal or whether the same lymphokines activate macrophages to inhibit different intracellular pathogens. A key to understanding these differences is understanding the lymphokines responsible for the activation. This has been frustrated by the number of lymphokine activities found in culture supernatants, even in cloned cell lines. Recently, human gamma interferon (IFN), a lymphokine that has been shown to have microbicidal activity (27, 31, 36) and has been associated with tumoricidal activity (19, 24, 33), has become available in recombinant form. This has enabled us to examine a lymphokine activity removed from other possible contaminating lymphokines. We describe here an examination of the ability of human monocyte-derived macrophages to be activated with human gamma IFN to kill tumors and intracellular mycobacteria and leishmania.

MATERIALS AND METHODS

Tubercle bacilli. Our laboratory strain of *Mycobacterium tuberculosis* Erdman was used (11). Thin-veil growth from cultures grown on potato-Kirchner medium (21) was subcultured in Middlebrook 7H9 liquid medium (Difco Laboratories, Detroit, Mich.) to obtain dispersed bacilli for infection. In these experiments, we used frozen stock cultures of bacteria in 7H9 medium. For freezing, the mycobacteria were grown in 75 to 100 ml of 7H9 medium in 125-ml culture flasks with stirring at 37°C until a concentration of 10^8 bacteria per ml was obtained. Growth curves indicated that the cultures were growing exponentially. Of this bacterial suspension, 3 ml was transferred into 5-ml sterile Nunc cryotubes (GIBCO Laboratories, Grand Island, N.Y.) and stored at −70°C until needed for infection.

Leishmania. *Leishmania donovani* promastigotes were grown in modified minimal essential medium as previously described (3). Cultures were inoculated at 2.5×10^6 cells per ml and cultured for 6 days at 25°C. Promastigote cultures became stationary by day 3. Only cultures that have been stationary for at least 3 days consistently infect and grow intracellularly (32).

Interferon. Recombinant human gamma IFN (rIFN-γ) was the gift of Genentec, Inc. The original solution was diluted to 10^5 U/ml in physiologic phosphate buffer (pH 6.0) containing 5 mg of human serum albumin per ml and stored at −90°C until needed. Before use, the rIFN-γ was diluted in RPMI 1640 medium (GIBCO) containing 50 mg of human serum albumin per ml (RPMIHSA5).

Isolation and culture of human peripheral blood mononuclear cells. Peripheral blood mononuclear cells were isolated on Ficoll-Hypaque by using a technique similar to that first described by Boyum (6) and modified by us (11). Routinely, 30 ml of venous blood was drawn from a purified protein derivative-negative (PPDN) individual, and 19 ml was mixed with 1 ml of Hanks balanced salt solution (HBSS) without

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Ca^{2+} or Mg^{2+}, containing 75 U of preservative-free heparin per ml. Each 10 ml of this mixture was layered onto 8 ml of a solution of 9% Ficoll 400 (Pharmacia Fine Chemicals, Piscataway, N.J.) and 33.3% Hypaque (Winthrop Laboratories, Div. Sterling Drug Inc., New York, N.Y.) in a sterile, 50-ml conical polypropylene centrifuge tube. This was centrifuged at 400 × *g* for 35 min at 4°C. Unheparinized blood (10 ml) was clotted to produce serum, and the serum was frozen at −70°C until used. After centrifugation, the monocytic cells were collected from the fluid interface and washed once by centrifugation for 10 min at 400 × *g* in HBSS without Ca^{2+} or Mg^{2+} containing 7.5 U of heparin per ml; then they were washed three times in HBSS without Ca^{2+} or Mg^{2+} and without heparin but with 117 mg of disodium EDTA per liter.

After the final washing, the cells to be infected were suspended at 10^7 cells per ml in RPMI 1640 medium with 1% unheated autologous serum, 2 mM L-glutamine, and 50 U of penicillin G (RPMINHS1) per ml. This concentration of penicillin does not affect Erdman replication either intra- or extracellularly (unpublished observation). Three 50-μl droplets of cell suspension were plated in 35-mm petri dishes (Falcon 1008; Becton Dickinson Labware, Oxnard, Calif.). For each group, plates were prepared in duplicate (six spots per group). The dishes were incubated at 37°C in a humidified atmosphere at 7.5% CO_2 in air for 30 min. After incubation, the drops were aspirated and discarded, and the plates were washed twice with HBSS (GIBCO) that was warmed to 37°C, to remove nonadherent cells. After being washed, 1.5 ml of RPMINHS1 was added per plate. The medium had been preincubated at 37°C in 7.5% CO_2 to adjust its temperature and pH. Adherent monocytes were cultured for 7 days before infection.

For experiments assaying tumoricidal activity, the monocytic cells were suspended in RPMINHS1 at various concentrations and plated in 0.5-ml volumes for 30 min in 24-well tissue culture dishes. The wells were washed with warm HBSS to remove nonadherent cells, and the adherent cells were cultured in 1 ml of RPMINHS1 for 7 days before target cells were added.

Assay for macrophage tumoricidal activity. The procedure used was similar to one initially described by Roberts and Vasil (30). Mouse L929 cells were grown in Falcon 3013 tissue culture flasks (Becton Dickinson) in 10 ml of RPMI 1640 containing 5% fetal calf serum (GIBCO), 2 mM L-glutamine, and 50 U of penicillin G per ml (RPMIFCS). Before use (3 days), the cultures were split by trypsinization. At 24 h after trypsinization, 5 ml of the medium from a flask was removed, and methyl-[^{3}H]thymidine (6.7 Ci/mmol; New England Nuclear Corp., Boston, Mass.) was added at 1 μCi/ml. The cells were labeled overnight, and the medium was removed and replaced with 10 ml of fresh RPMIFCS. After another overnight incubation, the cells were trypsinized and washed six times by centrifugation in HBSS to remove free label. The labeled L929 cells were then suspended in RPMIFCS at 10^5 cells per ml. The supernatant was removed from the macrophage cultures, and 1-ml volumes of L929 cells were added to each well.

At forty-eight hours after targets and macrophages were combined, 5 μl of a solution (1 mg/ml) of pancreatic DNase (Sigma Chemical Co., St. Louis, Mo.) in RPMI 1640 was added to each well. The dishes were incubated for 20 min at 37°C in 7.5% CO_2. The supernatants were then removed, added to 9 ml of Biofluor (New England Nuclear), and counted with a liquid scintillation spectrometer. Results were analyzed statistically with Student's *t* test.

Infection of macrophage cultures with tubercle bacilli. A frozen 7H9 medium stock culture of Erdman was thawed and sonicated for 75 s with a microprobe on an Ultrasonics, Inc., sonicator (Heat Systems, Plainview, N.Y.) with the output control at 2.5. The suspension was then diluted to 5 × 10^6 bacteria per ml (1/20 dilution) in RPMI 1640 containing 5% unheated autologous serum. The culture medium was removed from the 7-day macrophage cultures and replaced with 1.5 ml of the bacterial suspension per dish. The multiplicity of infection cannot be accurately determined with this procedure, even though the total numbers of macrophages and bacteria added to the cultures are known, because the bacteria are distributed throughout the plate, whereas the macrophages are localized in three individual spots on the plate (see illustration in reference 11). Macrophages were infected for 30 min at 37°C. The infection medium was removed, and the plates were washed three times with HBSS to remove extracellular bacteria. Then 1.5 ml of fresh RPMINHS1 was added to each plate.

Infection of macrophage cultures with leishmania. The procedure for infection of macrophages with promastigotes was adapted from procedures described by Sacks and Perkins (32) and Murray and Cartelli (22). It takes advantage of the fact that phagocytosed promastigotes transform into replicative, obligate intracellular amastigotes. Extracellular promastigotes cannot survive under the culture conditions described here.

The medium was removed from macrophage cultures 2 h before infection and replaced with 1.5 ml of glucose-free Krebs-Ringer phosphate buffer, pH 7.4 (35). Glucose starvation was used to decrease macrophage oxidative activity and to suppress oxygen-dependent killing of leishmania during phagocytosis (22). *L. donovani* promastigotes were diluted to 10^7/ml with Krebs-Ringer phosphate buffer, the buffer was removed from macrophage cultures, and 1.5 ml of the infecting suspension was added per plate. The plates were infected for 2 h at 37°C and 7.5% CO_2. After infection, the medium was removed, and the plates were washed twice with HBSS. Fresh RPMINHS1 (1.5 ml) was added back to each plate. The RPMINHS1 medium used after infection with leishmania was made with heat-inactivated autologous serum to prevent the possible killing of the leishmania by heat-labile components of normal human serum (13).

Assay for mycobacterial replication by using counts of AFB. Immediately following and at various times after infection, macrophage cultures were fixed with glutaraldelyde and stained with Ziehl-Neelsen as described previously (11). The numbers of intracellular bacteria were determined by counting acid-fast bacilli (AFB) under oil immersion at ×1,000 magnification. Macrophages were classified as containing 0, 1, 2 to 5, 6 to 20, or greater than 20 AFB. The bacteria in macrophages containing more than 20 AFB could not be counted accurately; therefore, the limit of counting was placed here. These categories were then given the values of 0, 1, 3.5, 13, and 30, respectively. The mean numbers of AFB per infected macrophage were calculated from these counts. Generally, 100 cells per spot were counted. Because each group had duplicate plates and each plate had three spots, values reported represent the mean of 600 cells. Differences between groups were analyzed with Student's *t* test.

Assay for mycobacterial replication by using counts of viable bacteria. The technique we used was adapted from one originally described by Biroum-Noerjasin for listeria (5). Immediately following and at various times after infection, the plates were agitated to resuspend the bacteria, and the

medium was removed from infected plates and saved. A lysing solution containing 1.1 ml of 7H9 medium and 0.4 ml of 0.25% sodium dodecyl sulfate (in physiologic phosphate buffer) was added to each plate. The plates were gently swirled from time to time during 10 min of incubation at room temperature. The lysates were then transferred to tubes containing 0.5 ml of 20% bovine serum albumin to neutralize the sodium dodecyl sulfate. Cell lysates and supernatants were sonicated for 15 s (power output of 2.5) to disperse bacterial clumps. Previous experiments indicated that bacterial viability was not affected either by the sodium dodecyl sulfate or by the sonication.

After sonication, the lysates and supernatants were serially diluted, and dilutions were plated on 7H10 agar (Difco) in plastic petri dishes (100 by 15 mm). Generally, four 15-μl spots were plated per dilution per plate. The spots were allowed to absorb onto the surface of the 7H10 plates at room temperature to prevent them from running together. The CFU were counted from plates cultured for 2 weeks in a humidified atmosphere of 7.5% CO_2 at 37°C. Duplicate 7H10 plates were prepared for each macrophage culture. Values for the groups were compared by using the two-sample z test.

To estimate the number of macrophages in each culture, matched plates were lysed with nuclear counting solution by using the technique described by Nakagawara and Nathan (26). Culture medium was removed from the plates and replaced with 1 ml of a counting solution consisting of 0.1 M citric acid, 1% Triton X-100, and 0.05 g naphthol blue black per 100 ml, adjusted to pH 2.2 with 1 M sodium hydroxide. The plates were allowed to stand at room temperature for 10 to 15 min. The lysate was disaggregated by repeated pipetting with a pipettor and disposable tips, and the nuclei were counted with a hemacytometer.

Assay for leishmanial replication. Macrophage cultures infected with leishmania were fixed for 10 min with methanol immediately following and at different times after infection. The cultures were then stained with Dif-Quick (American Scientific Products, McGaw Park, Ill.), and the numbers of intracellular amastigotes were counted at ×1,000 magnification under oil immersion. Each value represents counts on duplicate plates and between 400 and 600 macrophages. Statistical significance was determined with Student's t test.

Assay for mycobacterial growth in 7H9 medium. Frozen 7H9 stock cultures of mycobacteria were thawed and sonicated for 75 s as described above. An inoculum of the stock culture was added to 5 ml of 7H9 medium in screw-capped polystyrene tubes (16 by 125 mm) (Falcon), and the tubes were incubated on a slant at 37°C. Each day the tubes were vortexed, and the turbidity was determined with a Klett-Summerson colorimeter (Technical Equipment Corp., Denver, Colo.). The numbers of bacteria were read from a previously prepared standard curve relating bacterial concentration with turbidity units.

RESULTS

rIFN-γ-induced tumoricidal activity. To assess the tumoricidal activity of rIFN-γ-activated macrophages, various numbers of monocyte-derived macrophages cultured for 6 days were incubated overnight with final concentrations of 5, 50, or 500 U of rIFN-γ per ml and overlaid with L929 targets. rIFN-γ activated macrophages to kill tumor cells in a 48-h cytotoxicity assay, while having no effect on the targets themselves (Fig. 1).

Test for rIFN-γ-induced antimycobacterial activity. The 6-day monocyte-derived macrophages were cultured overnight with rIFN-γ as described for the tumoricidal assay above and then were infected on day 7 with tubercle bacilli. Fresh rIFN-γ was added to the infected cultures immediately after infection and every 2 days thereafter.

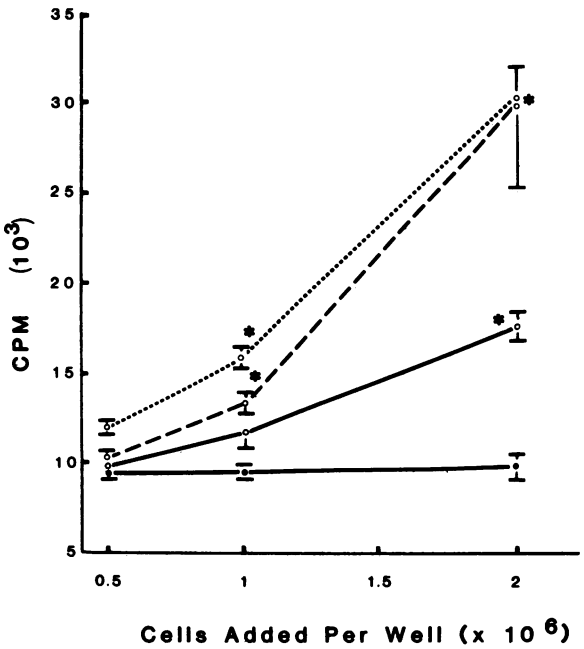

FIG. 1. rIFN-γ-induced tumoricidal activity. Peripheral blood mononuclear cells were added to wells at the concentrations indicated, washed to remove nonadherent cells, and cultured for 6 days. RPMIHSA5 (—●—) or rIFN-γ at final concentrations of 5 (—○—), 50 (– –○– –), or 500 (- - - -○- - - -) U/ml was added to triplicate wells. At 24 h later, [^{3}H]thymidine-labeled L929 target cells were added to each well along with fresh rIFN-γ. After 48 h, the supernatants were harvested and counted. Spontaneous release of label from L929 target cells without macrophages was 4,182 + 243. [^{3}H]thymidine release from L929 targets without macrophages but with 5, 50, or 500 U of rIFN-γ per ml was 3,847 + 36, 3,729 + 178, and 4,051 + 191, respectively. *, $P < 0.025$.

rIFN-γ activation had two effects on the macrophages as determined by AFB counts. The first was to decrease the percentage of macrophages initially infected (Fig. 2B). This decrease was consistently seen with PPDN cells. However, the extent of inhibition varied from experiment to experiment (see also Fig. 5B). The second effect was to increase the replication of the mycobacteria (Fig. 2A) and at the same time to increase the number of infected cells over 5 days of culture (Fig. 2B). We believe that the increase in the numbers of AFB per infected cell and in the percentage of infected cells was at least partially the result of the release of mycobacteria into the supernatant by the lysis of infected cells and the subsequent uptake of the released mycobacteria by other macrophages. This was suggested by the decrease in the number of adherent cells per plate with increasing rIFN-γ concentration (Table 1) and by the increase in extracellular mycobacteria. Significant extracellular replication of the mycobacteria in the absence of macrophages does not occur under our culture conditions (12), and rIFN-γ did not affect mycobacterial growth in bacteriologic culture medium (Fig. 3).

Experiments in which infected macrophages were lysed and viable bacteria determined supported results obtained with acid-fast counts (Table 1). In experiment 1, rIFN-γ pretreatment led to a small decrease in the initial infection at

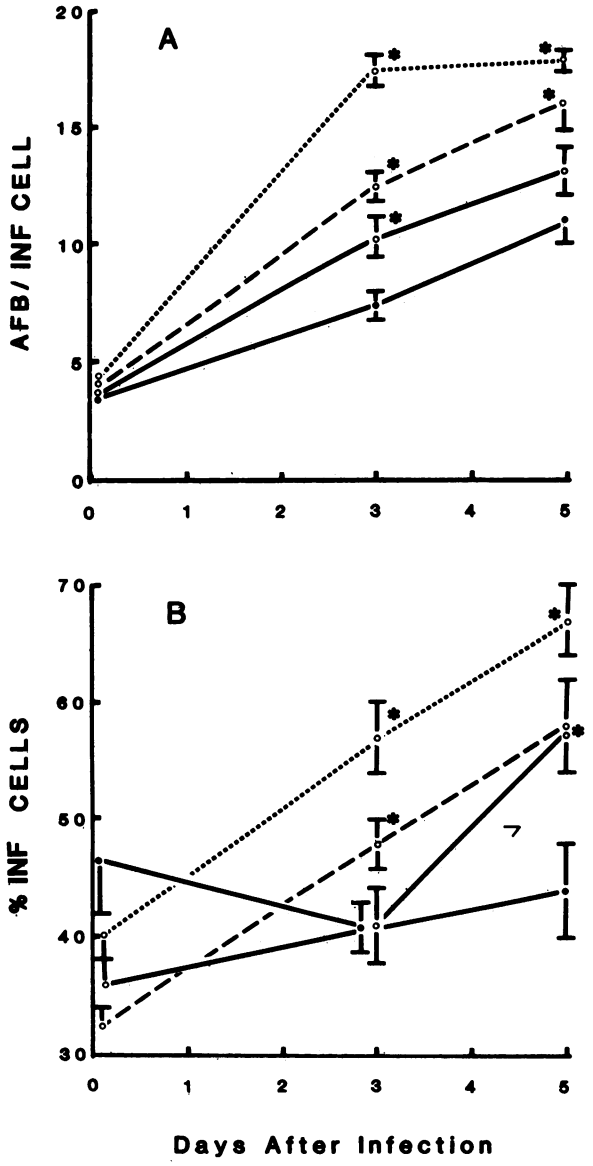

FIG. 2. Test for rIFN-γ-induced antimycobacterial activity: AFB counts. Adherent peripheral blood mononuclear cells were cultured for 6 days, and duplicate plates were treated with RPMIHSA5 (—●—) or with final concentrations of rIFN-γ of 5 (—○—), 50 (– –○– –), or 500 (- - -○- - -) U/ml for 24 h. The macrophage cultures were then infected with mycobacteria, and rIFN-γ was added immediately after infection and every 2 days thereafter. Cultures were fixed, and the numbers of AFB per infected macrophage (A) and the percent infected cells (B) were determined at the times indicated. *, $P < 0.05$. INF, Infected.

5 and 50 U/ml. Maximum inhibition of bacterial uptake occurred with 50 U/ml, similar to the results reported in Fig. 2.

In experiment 2 (Table 1), the infecting concentration of mycobacteria was decreased from 5×10^6 to 2.5×10^6 bacteria per ml. Once again, there was a slight decrease in the numbers of macrophage-associated mycobacteria in the rIFN-γ-pretreated cultures immediately after infection. In both experiments, the total numbers of mycobacteria in rIFN-γ-treated cultures were greater than in untreated controls.

The numbers of adherent cells in cultures treated with rIFN-γ showed a significant drop below control levels 5 to 7 days after infection. Both infection and rIFN-γ toxicity seemed to be responsible for the drop in cell numbers, since there was a loss of untreated cells after infection (Table 1) and when the cells were rIFN-γ treated and not infected (Table 2).

rIFN-γ-induced leishmanicidal activity. To determine whether we could activate macrophages for intracellular killing, we tested for rIFN-γ-induced macrophage microbicidal activity with a second intracellular pathogen, *L. donovani*. Macrophage cultures were pulsed with rIFN-γ on days 4 and 6 of culture, on day 7 immediately after infection, and every other day thereafter. Earlier experiments had indicated that a 24-h culture with rIFN-γ could activate macrophages against leishmania, but higher levels of anti-leishmania activity could be seen with cultures activated for 3 days.

rIFN-γ pretreatment had effects opposite those seen with mycobacterial (Fig. 4). A greater percentage of rIFN-γ-treated cells than control cells were infected with leishmania. Electron micrographs indicated that the promastigotes were intracellular and not simply surface associated (data not shown). This again was a consistent observation with PPDN cells, as was the maximum effectiveness at 50 U of rIFN-γ per ml. rIFN-γ activation of macrophages also caused both an inhibition of leishmania replication (Fig. 4A) and amastigote killing. Killing is best illustrated by the drop in the percent infected cells in cultures containing 50 U of rIFN-γ per ml (Fig. 4B). Examination of the infected macrophages with the electron microscope showed extensive promastigote destruction immediately after infection in rIFN-γ-treated macrophages.

As with mycobacteria, at high rIFN-γ concentrations the numbers of adherent cells appeared to decrease. However, unlike results with mycobacteria, the leishmania which might have been released by the lysis of rIFN-γ-treated macrophages were either noninfectious or were destroyed during phagocytosis, because the number of infected cells decreased with time in culture.

Parallel cultures incubated with 500 U of rIFN-γ per ml for 3 days before infection and infected with tubercle bacilli exhibited a decrease in uptake and an increase in bacillary replication (Fig. 5) similar to that which was seen when macrophages were preincubated for only 24 h. In other experiments, we have varied the time of incubation of the macrophages with rIFN-γ and the length in culture of the adherent cells before rIFN-γ treatment and infection and have been unable to demonstrate any antimycobacterial activity (data not shown).

DISCUSSION

IFN-γ is an immune lymphokine of broad specificity. In addition to its antiviral properties, it induces the expression of histocompatibility antigens on macrophages (17) and increases the production of reactive oxygen species (27). In both the mouse and human systems, human gamma IFN activity correlates with MAF activity (4, 19, 33, 42) and with the ability to activate macrophages, fibroblasts, and endothelial cells to interfere with the replication of a number of intracellular pathogens (27, 31, 36, 40). In these experiments, we tested rIFN-γ for its capacity to activate human macrophages to kill tumor cells and to interfere with the replication of two phylogenetically distinct intracellular pathogens, *M. tuberculosis* and *L. donovani*. The problems

TABLE 1. Counts of viable tubercle bacilli from rIFN-γ-treated macrophages[a]

Expt no. and day after infection	rIFN-γ concn/plate (U/ml)	No. of macrophages/plate[b] × (10^4)	Mycobacteria/plate[a]		Total bacteria	Fold increase
			Cells (×10^4)	Supernatant (×10^4)		
1						
0	0	30	26 (3)	1 (0.1)	27 (3)	
0	5	33	23 (0.1)	1 (0.1)	24 (0.1)	
0	50	31	20 (1)	1	21	
0	500	32	31 (8)	1 (0. 3)	32 (8)	
5	0	20 (2)	148 (3)	26 (5)	174 (6)	6
5	5	21 (1)	162 (22)	43 (7)	205 (23)	9
5	50	9 (1)	135 (5)	63 (6)	198 (8)[d]	9
5	500	9 (1)	133 (13)	63 (3)	196 (13)	6
2						
0	0	16 (3)	5 (1)	0.4 (0.04)	5.4 (1)	
0	50	23 (1)	5 (1)	1 (0.1)	6 (1)	
7	0	10 (1)	96 (7)	43 (3)	139 (8)	26
7	50	7 (0.3)	53 (7)	226 (0.1)	279 (7)[d]	47

[a] Values in parentheses are standard error of the mean.
[b] Determined as described in the Materials and Methods.
[c] Number of mycobacteria either cell associated or in the culture supernatant.
[d] Significantly different ($P < 0.025$) from untreated control.

of contaminating lymphokines, a problem in many of the above studies, were avoided by using rIFN.

In these experiments, rIFN-γ had MAF activity and could activate macrophages to inhibit intracellular leishmania, similar to what has been seen with crude mouse spleen cell supernatants (34). MAF and microbicidal activity, however, do not always associate. For example, EL-4 cells stimulated with phorbol myristate acetate produce a 23,000-molecular-weight factor that induces killing only of extracellular targets (23). Concanavalin A-induced spleen cell supernatants reportedly contain two fractions that only activate macrophages to kill intracellularly (24). None of these fractions resembles IFN. Consequently, it appears that there may be several species of lymphokine capable of activating macrophages to become microbicidal, tumoricidal, or both.

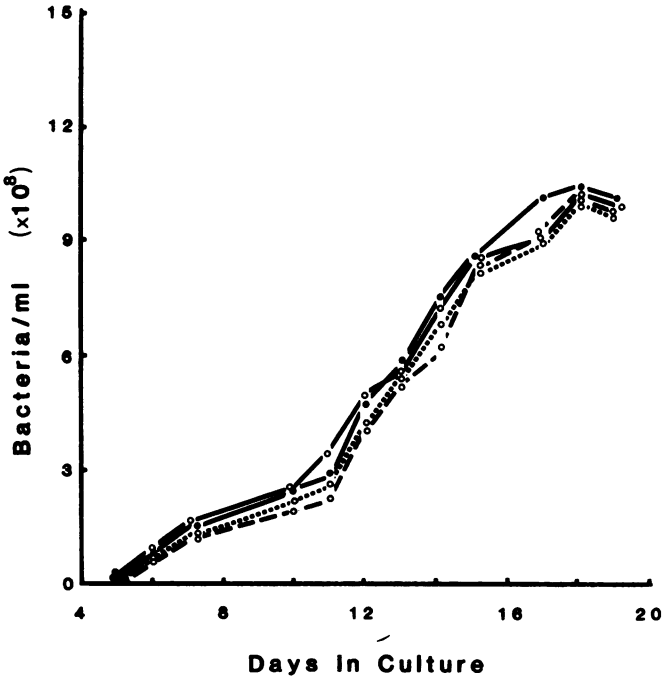

FIG. 3. The effects of rIFN-γ on the growth of mycobacteria without macrophages. Mycobacteria were inoculated into 5 ml of 7H9 medium at 17 × 10^4 bacteria per ml, approximately the concentration of bacteria in macrophage cultures immediately after infection. RPMIHSA5 (—●—) or rIFN-γ at final concentrations of 5 (—○—), 50 (– –○– –), or 500 (- - - -○- - - -) U/ml was added to triplicate tubes immediately after inoculation and every 2 days thereafter. The numbers of bacteria were determined turbidimetrically.

rIFN-γ pretreatment had opposite effects for mycobacteria and leishmania with regard to microbial uptake and replication. The number of organisms per infected cell was generally unaffected, yet there was an increase in the percentage of cells infected with leishmania and a decrease in mycobacterium-infected cells. Some investigators have found that lymphokine pretreatment has no effect on microbial uptake (5, 7, 9, 23, 28), whereas an increase in uptake of *Toxoplasma gondii* has been reported in lymphokine-pretreated human macrophage-like U937 cells (39). Conversely, a decrease in the number of infected cells as a result of lymphokine activation has been reported with *Legionella pneumophila* in human monocytes (14) and in the mouse with *Leishmania tropica* (25) and *Rickettsia* spp. (24, 37).

Macrophages, rIFN-γ activated to kill tumor cells or

TABLE 2. rIFN-γ-induced macrophage toxicity with uninfected cells[a]

rIFN-γ concn/plate (U/ml)	No. of macrophages/plate (×10^4) on culture day[b]	
	7	13
0	14.6 (1.9)	16.1 (0.1)
5	19.1 (0.1)	11.9 (0.6)
50	20.7 (2.3)	16.3 (1.4)
500	19.6 (2.6)	11.3 (1.0)

[a] Macrophages received rIFN-γ on day 6, on day 7 after a medium change, and every 2 days thereafter.
[b] Values in parentheses are standard error of the mean.

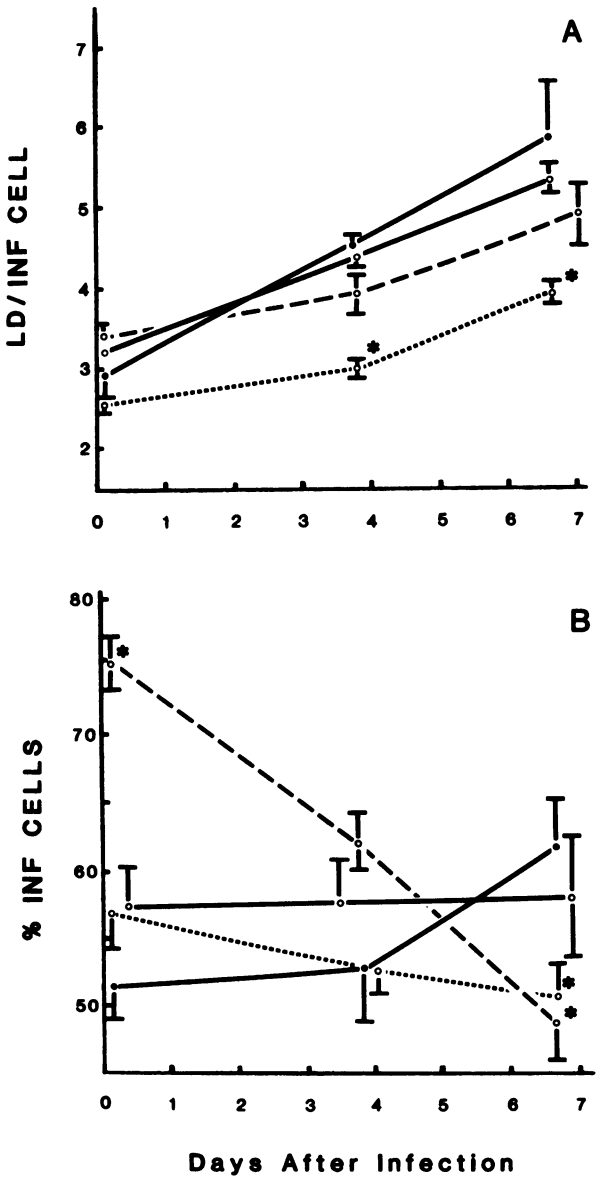

FIG. 4. rIFN-γ-induced leishmanicidal activity. Adherent peripheral blood mononuclear cells were cultured for 4 days, and on days 4 and 6 duplicate plates were treated with RPMIHSA5 (—●—) or with rIFN-γ at 5 (—○—), 50 (– –○– –), or 500 (- - -○- - -) U/ml final concentration. On day 7, the macrophage cultures were infected with *L. donovani* promastigotes, and rIFN-γ was added after infection and every 2 days thereafter. Cultures were fixed, and the numbers of *L. donovani* per infected cell (A) and the percent infected cells (B) were determined at the times indicated. *, $P < 0.05$; LD, *L. donovani*. INF, Infected.

leishmania, did not inhibit mycobacterial replication. High rIFN-γ concentrations actually enhanced it (Fig. 2 and 5, and Table 1). Enhanced mycobacterial growth by using whole spleen cell supernatants has been reported previously in mice (1).

The mycobacteria grew at an increased rate both intra- and extracellularly. Increased intracellular growth was evident as early as 3 days after infection as indicated by both AFB (Fig. 2A) and CFU counts (data not shown). Supernatants from rIFN-γ-treated and -infected macrophages were also capable of supporting enhanced extracellular growth (data not shown). Our preliminary experiments indicated that growth enhancement requires the interaction of both macrophages and lymphokine, because lymphokine alone (Fig. 3) or crude cell lysates do not induce enhanced growth (unpublished observations). The nature of the growth-enhancing factor is currently under investigation.

The possibility exists that we did not detect mycobacterial inhibition because of the assay system used. This seems unlikely because the same assay has been used for the preliminary identification of lymphokines that activate human macrophages to inhibit mycobacteria (11). These lymphokines, although somewhat elusive, do appear to be distinct from IFN-γ. This assay has also been used to determine the intramacrophage susceptibility of mycobacteria to streptomycin (12) and to investigate a natural mycobacteriostatic phase of human monocytes (manuscript

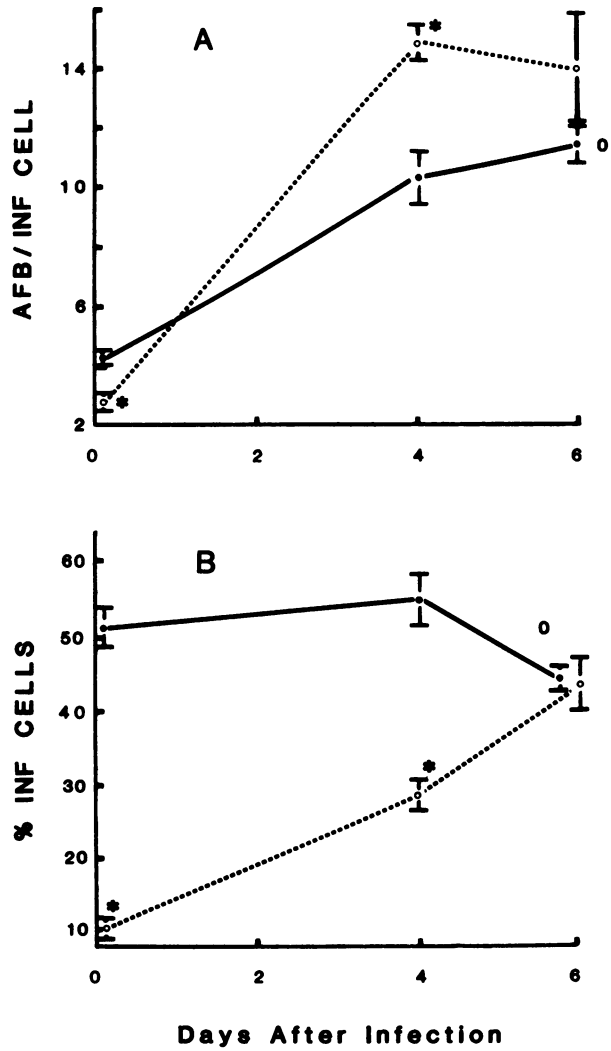

FIG. 5. Test for antimycobacterial activity in macrophage cultures pretreated with rIFN-γ for 3 days. In the same experiment as shown in Fig. 4, macrophage cultures were pretreated with RPMIHSA5 (—●—) or with a final concentration of 500 U of rIFN-γ per ml (- - -○- - -). The macrophages were infected with mycobacteria and counted as described in the legend to Fig. 2. (A) Number of AFB per infected cell; (B) Percent infected cells. *, $P < 0.005$. INF, Infected.

submitted for publication). A similar system has also been developed in this laboratory, showing that mouse macrophages can be lymphokine activated to inhibit mycobacterial replication (41). The relationship of this lymphokine to IFN-γ is currently under investigation. Consequently, it is more likely that rIFN-γ does not activate macrophages appropriately for antimycobacterial activity than that the system was unable to detect the activation.

The mechanisms of acquired immunity against mycobacteria and leishmania are thought to be similar, both involving the lymphokine-induced activation of macrophages to become microbicidal. The differences in the uptake and intracellular fate of these pathogens in rIFN-γ-activated macrophages suggest that the mechanisms of immunity may not be the same. The differences could lie in the populations of responding lymphocytes, in the lymphokines produced by them, and consequently, in the type of stimulation induced in macrophages. Different cell populations responsible for immunity and tissue damage have been suggested for infections caused by mycobacteria (10, 29), listeria (15, 16, 18), and leishmania (20). Thus, the appearance of cells producing human gamma IFN could exacerbate a tuberculous lesion by causing increased mycobacterial replication. This could account for the explosive mycobacterial replication commonly detected in the caseous lesions of tuberculosis (8). Alternatively, a second population of cells could elaborate a lymphokine that induces the intracellular destruction of mycobacteria. Human gamma IFN-producing cells would be beneficial at the site of infection with leishmania.

The evidence presented here and elsewhere (34) that lymphokine-activated macrophages can inhibit some intracellular pathogens but not others suggests that there may be subpopulations of cells and lymphokines that activate macrophages in different ways. It must be considered at this time, though, that some differences in microbe survival may be caused by variations in laboratory techniques. The cloning of lymphocyte populations and the cloning and dissemination of lymphokines, as has been done with IFN-γ, should help provide more definitive answers.

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