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Preservation of Monocyte Effector Functions against *Mycobacterium avium-M. intracellulare* in Patients with AIDS

JOHN L. JOHNSON,[1] HIROE SHIRATSUCHI,[1] HIROKAZU TOBA,[2] AND JERROLD J. ELLNER[1]*

Department of Medicine, Case Western Reserve University, University Hospitals, and Cleveland Veterans Administration Medical Center, Cleveland, Ohio 44106,[1] and the Osaka Prefectural Habikino Hospital, Osaka 583, Japan[2]

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***Mycobacterium avium-M. intracellulare* is a frequent cause of late disseminated infection in patients with AIDS. The ability of human peripheral blood monocytes to phagocytose and kill *M. avium* was examined in an in vitro model. Monocytes were obtained from 13 healthy volunteers and 11 patients with AIDS, three of whom had documented disseminated *M. avium* infection. Monocytes were precultured for 2 days before infection with two AIDS-associated and two non-AIDS-associated strains of *M. avium*. Uptake of *M. avium* as measured by counting intracellular acid-fast bacilli did not differ among healthy subjects, patients with AIDS, or patients with AIDS and previously documented disseminated *M. avium* infection. Intracellular growth of *M. avium* was examined by a CFU assay of cell lysates from *M. avium*-infected monocytes after 0, 4, and 7 days of culture. Intracellular growth inhibition of *M. avium* at 7 days after infection was comparable between patients with AIDS and healthy donors for all *M. avium* strains tested. The effects of the addition of recombinant gamma interferon on *M. avium* uptake and intracellular growth in monocytes also were studied. Pretreatment of monocytes with gamma interferon prior to infection suppressed monocyte phagocytosis of *M. avium*. Continuously coculturing of monocytes with gamma interferon after infection augmented killing of *M. avium* among both patients with AIDS and healthy controls for three of the four strains of *M. avium* tested. The magnitude of this effect, however, was variable from donor to donor and strain to strain. No significant differences were noted between the growth-inhibiting abilities of gamma-interferon-treated monocytes obtained from healthy volunteers and those obtained from patients with AIDS.**

Disseminated *Mycobacterium avium-M. intracellulare* infection is a common late opportunistic infection in patients with AIDS (12, 13, 17). Disseminated *M. avium* infection occurs as the initial AIDS-defining condition in 3 to 4% of AIDS cases (3), and evidence of disseminated *M. avium* infection has been present at the time of death in up to 47 to 56% of autopsied patients with AIDS (9, 42). This finding is in striking contrast to the rarity of clinically significant infection with this ubiquitous organism in immunocompetent hosts. In addition to increased numbers of disseminated *M. avium* infections in such patients, patients with AIDS also have an increased incidence of infection with other nontuberculous mycobacteria and tuberculosis (38).

Mononuclear phagocytic cells are key effector cells in the immune response to intracellular pathogens such as mycobacteria. Monocytes can be infected in vitro with human immunodeficiency virus (HIV) (14, 24), and blood monocytes and tissue macrophages are infected in vivo (15, 27, 32). Since only 1 to 5% of normal blood-derived monocytes/macrophages were directly infectible with HIV in vitro (14) and the level of viral replication appears to be lower in monocytes/macrophages than in lymphocytes, alterations in monocyte functional ability in patients with AIDS are likely to be indirectly mediated. Previous studies have documented normal effector functions of monocytes isolated from patients with AIDS, including oxidative burst generation and intracellular killing or inhibition of *Toxoplasma gondii* or *Chlamydia psittaci* (19), killing of aspergilli and cryptococci (43), phagocytosis of latex particles, expression of interleukin 1, and antibody-dependent cellular cytotoxicity against WEHI 164 sarcoma cells (26); defective monocyte accessory cell function for lymphocyte responses to soluble antigens (29) and mitogens (28) has, however, been reported recently. Selective impairment of macrophage chemotaxis to several chemoattractants, including lymphocyte-derived chemotactic factor and *N*-formyl-methionyl-leucyl-phenylalanine, in patients with AIDS also has been reported (26, 37). Conflicting data exist regarding monocyte effector function capability against mycobacteria in patients with AIDS; both normal (34) and decreased (4) abilities to limit intracellular *M. avium* growth have been described.

Gamma interferon (IFN-γ), which is a product of stimulated T cells, increases intracellular killing of *T. gondii* (18, 21, 23), *Leishmania* species (7, 25) and other pathogens (2, 8, 31). Whereas the addition of IFN-γ to murine macrophages enhances mycobacterial killing (10, 11), the effects of IFN-γ on mycobacterial killing by human monocytes are controversial. Investigators have shown that IFN-γ has variable effects on the intracellular growth of *Mycobacterium tuberculosis* in human monocytes and macrophages (7, 30, 40). Bermudez and Young (1) have shown that IFN-γ is ineffective in augmenting *M. avium* killing by human monocytes in vitro. Murray et al. have also recently demonstrated that, unlike lymphocytes from healthy tuberculin reactors or patients without AIDS but infected with *M. avium*, lymphocytes from patients with AIDS and disseminated *M. avium* infection fail to undergo blastogenesis when exposed to bacillus Calmette-Guérin (*M. bovis* BCG)- or *M. avium*-derived antigens and that such patients' lymphocytes secrete only very low levels of IFN-γ in response to these antigens (21). Monocytes and tissue macrophages from patients with AIDS are, however, fully responsive to the effects of IFN-γ in vitro; stimulation with this cytokine leads to augmented release of hydrogen peroxide after exposure to phorbol myristyl acetate, and increased killing of *T. gondii* (predom-

* Corresponding author.

inantly oxygen-dependent killing) and growth inhibition of *C. psittaci* (predominantly oxygen-independent killing) (18, 19).

The purpose of this study was to examine the effector function of 2-day-precultured monocytes, a population of cells relatively nonpermissive to the growth of intracellular pathogens, from healthy subjects, patients with AIDS, and patients with AIDS and known concurrent disseminated *M. avium* infection in an in vitro model of *M. avium* infection. We found comparable abilities of monocytes from healthy subjects and patients with AIDS to ingest and limit the intracellular growth of several AIDS- and non-AIDS-associated strains of *M. avium* and to respond to IFN-γ.

MATERIALS AND METHODS

Human subjects. Eleven patients with AIDS (10 males and 1 female; 24 to 38 years old; mean age, 31 ± 5 years) were studied. Three patients with AIDS had known disseminated *M. avium* infection as documented by repeated positive blood cultures prior to the time of study. None of the patients was receiving antimycobacterial drug therapy at the time of study. Seven of the patients had previous *Pneumocystis carinii* pneumonia (four within 4 weeks prior to study). Three of the patients were completing acute treatment for *Pneumocystis* pneumonia with trimethoprim-sulfamethoxazole or trimethoprim-dapsone at the time of study. Two patients were receiving trimethoprim-sulfamethoxazole or trimethoprim-dapsone as secondary prophylaxis against recurrent *Pneumocystis* pneumonia. All patients with AIDS except one (who had AIDS-related encephalopathy as the AIDS-defining condition) had previously documented opportunistic infections usually with *P. carinii* or esophageal candidiasis. Three patients were receiving daily treatment with zidovudine. The clinical spectrum of the patients with AIDS studied ranged from recently diagnosed cases presenting with an initial opportunistic infection to late advanced disease. The time since the diagnosis of AIDS was established was 6.7 ± 7.7 months (mean ± standard deviation, with a range of 1 to 24 months). The group of healthy volunteers included 13 subjects (7 males and 6 females, 24 to 43 years old; mean age, 31 years) who were not in any group recognized to be at increased risk for HIV infection.

Microorganisms. Four *M. avium* strains were used in these experiments. Strains 86m2096 and LR542 were clinical isolates from patients with AIDS and disseminated *M. avium* infection. *M. avium* LR542 and LR114 (parent strain) were kindly donated by J. F. Crawford, John L. McClellan Memorial Veterans Hospital, Little Rock, Ark., and were serovar 4. Strain 86m2096 was a clinical isolate from University Hospital, Cleveland, Ohio. Strains LR114F and LR114R have flat, transparent and round, opaque colonial morphologies and were separated on this basis from a non-AIDS isolate. Strains LR114F and 86m2096 were highly virulent strains as defined by rapid intracellular growth and short dividing times in previous experiments, whereas strains LR542 and LR114R were of low virulence or relatively avirulent (36, 41). Strains of *M. avium* were grown in Middlebrook 7H9 broth supplemented with ADC enrichment (Difco Laboratories, Detroit, Mich.) at 37°C and 5.0% CO_2 in air. When the bacterial density was approximately 1×10^8 to 5×10^8/ml (log-phase growth), the mycobacteria were aliquoted into freezing tubes (Nunc, Copenhagen, Denmark) and stored at −70°C until use. Before infection, bacteria were thawed in a 37°C water bath and sonicated for 5 s with a Micro-Ultrasonic cell disrupter (Kontes, Vineland, N.J.) to obtain a single cell suspension and were diluted to a density of 10^7 bacteria per ml in RPMI 1640 medium (Whittaker M. A. Bioproducts, Walkersville, Md.) supplemented with 5% autologous unheated serum.

Isolation and culture of monocytes. Heparinized venous blood and sera were obtained from patients with AIDS, described above, and healthy volunteers. Isolation and culture of monocytes were performed as described previously (35, 36, 41). Peripheral blood mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation (Pharmacia, Piscataway, N.J.) and were suspended at a final density of 10^7 cells per ml in RPMI 1640 (with 2 mM L-glutamine, 15 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES] buffer, and penicillin G [100 U/ml]) supplemented with 2% autologous unheated serum. Three 50-μl droplets of a fresh peripheral blood mononuclear cell suspension were placed in each 35-mm petri dish (no. 1008; Falcon, Oxford, Calif.), and the dishes were incubated for 60 min at 37°C. Nonadherent cells were removed by gently washing the plates twice with prewarmed medium. The monocytes were precultured for 2 days in 2 ml of RPMI 1640 medium supplemented with 2% autologous unheated serum at 37°C and 7.5% CO_2 in air before infection. For infection, monocytes were incubated with 1.5 ml of bacterial suspension (10^7 bacteria per ml in medium containing 5% autologous serum) for 60 min at 37°C. The dishes were washed four times with prewarmed medium to remove extracellular bacteria and were cultured in 2 ml of medium containing 2% autologous serum for up to 7 days in the presence or absence of 10 to 1,000 U of recombinant human IFN-γ (Amgen Biochemicals, Thousand Oaks, Calif.) per ml. Representative plates infected with each strain of *M. avium* were fixed in methanol immediately after infection and washing for assessment of uptake of *M. avium*. Samples were harvested for CFU assay immediately after infection and at 4 and 7 days after infection.

The numbers of monocytes in representative plates were determined before the 2-day preculture period, immediately after infection (day 0), and at 4 and 7 days after infection by the method of Nakagawara and Nathan (22). The number of adherent monocytes decreased approximately 30% over the 2-day preculture period before infection. Approximately 1.5×10^5 to 2×10^5 monocytes per plate were present following the next 7 days of culture.

Counting of AFB. Immediately following infection, representative plates were fixed with methanol for 10 min and stained by Kinyoun's modified acid-fast stain method. The number of intracellular bacteria was determined by counting the number of acid-fast bacilli (AFB) within monocytes by light microscopy at ×1,000 magnification under oil immersion. A total of 600 to 900 consecutive monocytes was examined, 200 to 300 monocytes from each of the three spots on the petri dish. Semiquantitative scoring of the numbers of AFB was done by the method of Crowle et al. (5). The percentage of monocytes infected and the number of bacilli per 100 monocytes were calculated, and the data are reported as means ± standard errors.

CFU assay. The CFU assay was performed as described previously (35, 36, 41). Briefly, infected monocyte cultures in 35-mm-diameter petri plates were harvested immediately after infection (day 0) and 4 and 7 days after infection. Samples were stored at −70°C until CFU assay was performed. Monocyte lysates for enumerating intracellular *M. avium* were prepared by adding 1.1 ml of Middlebrook 7H9 medium and 0.4 ml of 0.25% sodium dodecyl sulfate (SDS) in phosphate-buffered saline to each petri plate. The plates

were incubated at room temperature for 10 min. The lysates were then transferred to tubes containing 0.5 ml of 20% bovine serum albumin to neutralize the SDS. Cell lysates were sonicated for 5 s to ensure a single cell suspension of mycobacteria and were serially 10-fold diluted in 7H9 medium. Three 10-μl aliquots of each dilution were plated on Middlebrook 7H10 agar supplemented with OADC (Difco) in 60-mm petri dishes (no. 1007; Falcon). The spots were allowed to adsorb onto the surfaces of the 7H10 plates at room temperature to prevent them from running together. The agar plates were then incubated at 37°C in a humidified atmosphere with 5% CO_2 for 5 to 7 days until visible colonies were present. The number of colonies at each dilution was counted with a stereomicroscope and averaged. The results were expressed as means ± standard deviations of CFU per ml or of $\log_{10}$ mean CFU per milliliter of cell lysate, which represented the bacterial CFU associated with approximately 10^5 monocytes.

The data were plotted semilogarithmically as $\log_{10}$ mean CFU per milliliter versus time (days after monocyte infection with *M. avium*) to demonstrate exponential bacillary replication and to facilitate calculation of bacterial dividing time by the method of Crowle et al. (5).

Statistical analysis. The significance of differences between groups was calculated by Student's *t* test. The significance of differences between cultures performed in the presence and absence of added recombinant IFN-γ was tested by the paired *t* statistic. The significance of differences in patterns of intracellular mycobacterial growth curves was calculated by the χ^2 statistic.

RESULTS

Monocyte survival in in vitro culture. Serial counts of adherent monocytes were performed on representative plates after 0 to 7 days of culture. During the subsequent 7-day culture period, monocyte numbers decreased by 22 to 30%. The initial number of monocytes present and the numbers remaining after 4 and 7 days of culture were comparable between patients with AIDS and healthy donors [$(2.3 \pm 0.2) \times 10^5$ versus $(1.7 \pm 0.2) \times 10^5$ monocytes per plate on day 0 and $(1.7 \pm 0.2) \times 10^5$ versus $(1.8 \pm 0.2) \times 10^5$ monocytes on day 7]. Monocyte numbers in plates cultured with IFN-γ also were comparable between patients with AIDS and healthy donors [$(1.6 \pm 0.2) \times 10^5$ monocytes per plate for patients with AIDS versus $(1.6 \pm 0.3) \times 10^5$ monocytes per plate for healthy subjects on day 7].

Uptake of *M. avium* by human monocytes from healthy subjects and patients with AIDS. The uptake of *M. avium* was assayed by counting cell-associated mycobacteria stained with Kinyoun's modified acid-fast stain following infection of the cells with *M. avium* in vitro. This method has been widely used to study the uptake of mycobacteria by mononuclear phagocytes (5, 6, 7, 41). AFB staining, while semiquantitative, detects both living and nonviable *M. avium* cells phagocytosed by monocytes, reflecting the overall intensity of monocyte phagocytosis of the target more directly. The CFU assay detects living *M. avium* cells that have been phagocytosed by monocytes. The data from CFU assays on day 0 and direct AFB counts in fixed monocytes were consistent.

The uptakes of *M. avium* were comparable between healthy subjects and patients with AIDS for all four strains of *M. avium* tested (data not shown). In earlier experiments, we noted strain-to-strain differences in the number of AFB ingested by monocytes from healthy donors (36). The percentage of monocytes ingesting AFB was greater in experiments using *M. avium* strains with domed, opaque colonial morphology than in those using flat, transparent strains (36). Similar results were seen in these experiments using monocytes from patients with AIDS. The percentage of monocytes infected with AFB was 12% for the highly virulent flat LR114F strain compared with 34 to 44% for the other three strains tested.

Previous experiments in our laboratory involving infection of human monocytes with *M. avium* at 4 and 37°C to distinguish AFB ingested by monocytes versus AFB adherent to the cell surface showed that only a few cell-associated AFB were visible when monocytes were infected at 4°C (36). Similar phagocytosis experiments were performed with *M. avium* strains stained with Lucifer yellow dye. Analysis of these preparations by flow cytometry demonstrated very limited nonspecific adherence of the mycobacteria to the cells and demonstrated that most cell-associated mycobacteria had been phagocytosed (unpublished data). By using electron microscopy, Schnittman et al. also have shown that most cell-associated *M. avium* strains are ingested and not merely adherent (34).

Intracellular growth inhibition of *M. avium* in human monocytes from patients with AIDS. We examined the ability of monocytes obtained from patients with AIDS to limit the intracellular growth of four *M. avium* strains of differing colonial types and origins in our in vitro system. Strains 86m2096 and LR114F were virulent and strains LR542 and LR114R were relatively avirulent in previous experiments using monocytes from healthy donors (36). Monocytes precultured for 2 days were infected with four strains of *M. avium* and were cultured for up to 7 days. The CFU assays were performed on samples harvested immediately and 4 and 7 days after infection, and the data are shown in Fig. 1. Intracellular *M. avium* CFU on day 0 were comparable between patients with AIDS and healthy subjects. Enhanced bacteriostasis of strain LR542 was exhibited in monocytes of patients with AIDS (6 of 10 patients) compared with that in monocytes of healthy donors (0 of 8 donors, $P < 0.05$, χ^2 test). Intracellular mycobacterial dividing times of strain LR114F appeared to be longer and more variable in patients with AIDS (34.4 ± 8.8 h; range, 11 to 83 h) than in healthy donors (14.4 ± 0.8 h; range, 10 to 21 h). There was no significant difference in intracellular *M. avium* growth inhibition between patients with AIDS and disseminated *M. avium* infection and the other patients with AIDS.

The intracellular growth curves of three of four *M. avium* strains showed a biphasic pattern with initial killing evident at 4 days after infection followed by multiplication and outgrowth of the surviving organisms. Strain LR114F multiplied between 0 and 4 days in monocytes of both patients with AIDS and healthy donors tested, with further acceleration of growth after 4 days in culture.

Modulation by IFN-γ of the uptake of *M. avium* by human monocytes obtained from patients with AIDS. Previously, we reported that IFN-γ inhibited phagocytosis of *M. avium* by monocytes from healthy donors (36, 41). We studied the effects of IFN-γ on *M. avium* phagocytosis by monocytes from patients with AIDS. Monocytes were allowed to adhere to plastic petri dishes and were precultured for 2 days in the presence (300 U/ml of culture media) or absence of human recombinant IFN-γ. This activity of IFN-γ was chosen as it had previously been shown to depress *M. avium* phagocytosis in cultured adherent monocytes obtained from normal healthy subjects (41). The monocytes were subsequently infected with the four different strains of *M. avium* used in

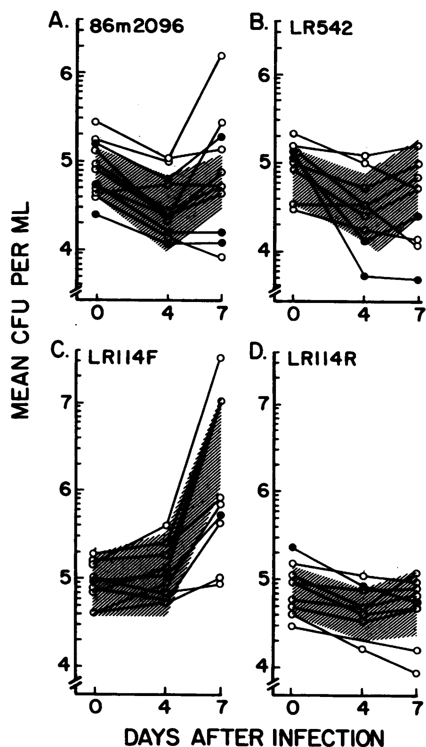

FIG. 1. Intracellular growth of four strains of *M. avium* in monocytes from patients with AIDS and healthy donors. *M. avium* growth curves in monocyte lysates from patients with AIDS but without known prior *M. avium* infection (○) and from patients with AIDS and prior known disseminated *M. avium* infection (●) are shown. Shaded areas represent the means ± 1 standard deviation of CFU of *M. avium* in monocyte lysates of all healthy donors.

this study. Uptake of *M. avium* was assayed by direct-light microscopic enumeration of cell-associated *M. avium* after Kinyoun staining. Pretreatment of monocytes derived from patients with AIDS with IFN-γ (300 U/ml) significantly decreased the percentage of monocytes infected with *M. avium* for all four strains of *M. avium* tested and decreased the number of AFB ingested per 100 infected monocytes for the two more virulent *M. avium* strains ($P < 0.05$, paired *t* test) (Table 1).

TABLE 1. Effects of IFN-γ on phagocytosis of *M. avium* by monocytes from patients with AIDS

M. avium strain	No. tested	Preculture condition before infection[a]	% Monocytes infected[b]	No. of AFB/100 monocytes[b]
86m2096 (virulent)	9	No IFN	39.5 ± 7.2]	170 ± 44]
	9	IFN	26.5 ± 3.3]	91 ± 14]
LR542 (avirulent)	7	No IFN	35.0 ± 6.0]	131 ± 31
	7	IFN	28.7 ± 5.3]	96 ± 19
LR114F (virulent)	8	No IFN	12.6 ± 3.6]	29 ± 10]
	8	IFN	8.3 ± 3.2]	16 ± 7]
LR114R (avirulent)	8	No IFN	34.2 ± 5.2]	126 ± 34
	8	IFN	26.8 ± 3.4]	78 ± 12

[a] The monocytes indicated were precultured with IFN-γ (300 U/ml) for 2 days prior to infection with *M. avium*.
[b] All data are expressed as means ± standard errors. Brackets indicate $P < 0.05$ from the paired *t* test.

TABLE 2. Effect of coculture with IFN-γ on intracellular *M. avium* growth in monocytes from patients with AIDS

IFN-γ dose (U/ml)	Mean CFU[a] of *M. avium* strain per ml in monocyte lysate on day 7 (10^4)		
	86m2096	LR542	LR114R
0	10.4 ± 4.1	4.6 ± 2.1	5.9 ± 2.8
300	7.8 ± 4.8	5.0 ± 1.9	2.5 ± 1.1
1,000	7.1 ± 3.4	5.3 ± 1.9	3.0 ± 1.2

[a] Data are expressed as means ± standard errors for monocytes from four patients with AIDS.

Modulation by IFN-γ of the intracellular growth of *M. avium* by peripheral blood monocytes from patients with AIDS. Monocytes from patients with AIDS and healthy subjects were precultured for 2 days and infected with four strains of *M. avium*. The infected monocytes were then cultured continuously in the presence (10 to 1,000 U/ml) or absence of human recombinant IFN-γ for up to 7 days. Representative plates were harvested immediately after infection (day 0) and at 4 and 7 days. Intracellular growth of *M. avium* was measured as the CFU count of *M. avium* in monocyte cell lysates at each respective time point after infection. Dose-response experiments showed that the optimal dose of IFN-γ causing intracellular *M. avium* growth inhibition was 300 U/ml for both patients with AIDS and healthy subjects. At suboptimal doses, IFN-γ augmented intracellular *M. avium* growth inhibition in a comparable and dose-dependent fashion for both patients with AIDS and healthy subjects (data not shown). No further increase in intracellular *M. avium* growth inhibition was seen when monocytes from patients with AIDS were cultured with higher doses of IFN-γ (1,000 U/ml) (Table 2).

Culturing infected monocytes continuously in the presence of IFN-γ (300 U/ml) decreased intracellular growth of *M. avium* at 4 or 7 days by 30 to 50% for patients with AIDS ($P < 0.05$ for three of four strains of *M. avium* tested, paired *t* test) (Fig. 2). Studies using monocytes from healthy donors were performed concurrently with the studies using monocytes from patients with AIDS. There were no significant differences between the effects of IFN-γ on intracellular *M. avium* growth in monocytes derived from patients with AIDS and in monocytes derived from healthy subjects (36). IFN-γ had no direct effect on *M. avium* growth in culture medium without monocytes (35).

DISCUSSION

This study shows that monocytes obtained from the peripheral blood of patients with AIDS have preserved effector cell function with regard to their ability to phagocytose and limit the intracellular growth of *M. avium* compared with monocytes derived from healthy donors. These data confirm the earlier data obtained by Schnittman et al. that monocytes from patients with AIDS exhibit capacities for *M. avium* uptake and killing comparable to those of monocytes from healthy individuals (34) but are in apparent conflict with the data of Crowle et al., who have recently reported that macrophages from HIV-infected patients are abnormally permissive in allowing intracellular *M. avium* growth in an in vitro culture system (4). There are several possible reasons for these differences. Our experimental system differed from that of Crowle et al. in certain important aspects. Crowle et al. used monocytes that were preincubated for 7 days prior

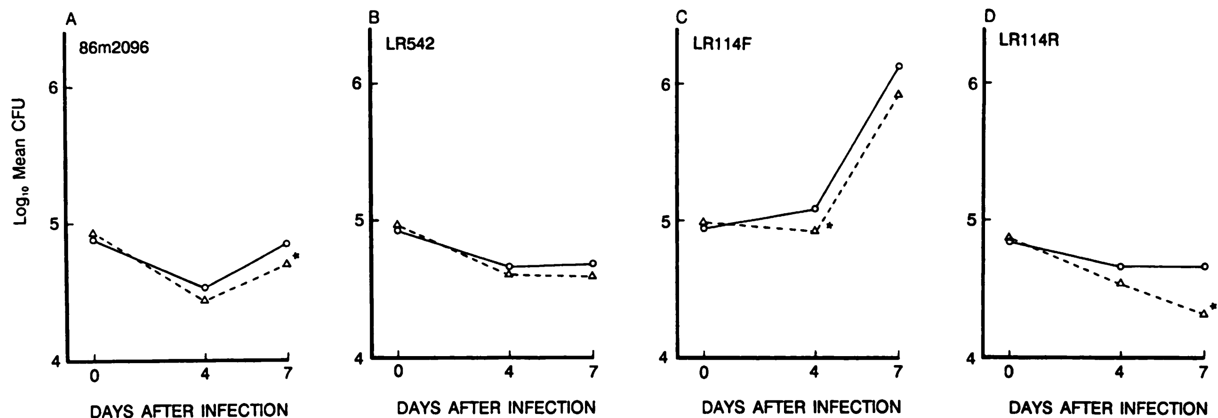

FIG. 2. Intracellular growth of four strains of *M. avium* in monocytes from patients with AIDS, cultured in the presence (300 U/ml) (△) or absence (○) of exogenous IFN-γ added to the culture medium after infection. Data are expressed as the $\log_{10}$ mean CFU of *M. avium* per milliliter in monocyte lysates at each time point from nine patients for strain 86m2096, seven patients for strain LR542, eight patients for strain LR114F, and eight patients for strain LR114R. Asterisks represent significant differences ($P < 0.05$, paired t test) in *M. avium* numbers between control cultures and IFN-γ-containing cultures.

to *M. avium* infection. Douvas et al. (6) have previously reported that monocytes precultured for 3 days prior to infection allowed less intracellular *M. tuberculosis* growth than did monocytes precultured for 7 days prior to infection, implying that a decline in this effector cell function occurs in normal monocytes after prolonged culture periods. We chose to use 2-day-precultured monocytes to allow adequate time to preculture cells with various stimuli but to avoid the decline in intracellular growth inhibition capacity noted by Douvas et al. in monocytes cultured for longer time periods (6). In addition to the different culture conditions used by Crowle et al., the infecting strains of *M. avium* used in these experiments were different; the differences in the results may be attributable to strain-to-strain differences in mycobacterial virulence (36).

It is important that the appearance and numbers of monocytes recoverable from the monolayers of cultured monocytes from HIV-infected patients and healthy subjects were comparable and remained so throughout the course of the experiments. Increased monocyte losses from the *M. avium*-infected monolayers in the patients with AIDS might otherwise have been interpreted as increased intracellular bacterial killing. The intensity of initial monocyte infection with *M. avium* as measured by the percentage of monocytes infected with AFB and the mean number of AFB per 100 monocytes also were comparable between patients with AIDS and healthy subjects, allowing direct comparisons of intracellular growth at later points during culture.

Our data and those of Schnittman et al. (34) are consistent with the hypothesis that the marked predilection of patients with HIV infection to *M. avium* disease may be related to factors extrinsic to the monocyte itself. The major identified immunologic defect in AIDS is the global destruction of $CD4^+$ (helper or inducer) lymphocyte populations, although secondary deficits occur in CD8 cells, natural killer cells, B cells, and macrophages. It is possible that disseminated *M. avium* disease in AIDS is due to impaired production of monocyte-activating factor activity from faulty T-cell function or other defects in signalling along the T-cell–cytokine–macrophage axis.

The role of IFN-γ in the control of intracellular mycobacterial infections remains controversial. IFN-γ is produced by T lymphocytes in response to specific antigenic stimulation. IFN-γ does not appear to be directly toxic to microbial pathogens but acts as a monocyte-activating factor to enhance microbial killing by host effector cells. Flesch and Kaufman (10) have demonstrated enhancement of macrophage growth-inhibitory capacity for *M. tuberculosis* by IFN-γ-pretreated murine bone marrow-derived macrophages although the effect was not present with all strains of *M. tuberculosis* tested. Rook et al. have shown bidirectional effects of IFN-γ on the intracellular replication of *M. tuberculosis* in human macrophages, with significant donor-to-donor variability (30). Bermudez and Young have reported that IFN-γ neither enhanced nor depressed the ability of 7-day-precultured human monocytes to limit *M. avium* growth (1). Some of the variability in the results is likely due to differences in the source and duration of preculture of mononuclear phagocytes, differences in virulence among *M. avium* strains, and interspecies differences in susceptibility to mycobacterial infection (33, 36).

Our data demonstrate that monocytes derived from patients with AIDS can be stimulated by a known macrophage-activating factor such as IFN-γ to augment the capability to limit intracellular growth of *M. avium*. These results were obtained by using doses of IFN-γ demonstrated to have optimal macrophage-activating factor activity in experiments with monocytes from patients with AIDS and healthy subjects (36). The absolute decrease in colony counts in the presence of IFN-γ was small but similar to that of monocytes from healthy subjects. In earlier experiments, Schnittman et al. demonstrated IFN-γ enhancement of intracellular *M. avium* growth inhibition by monocytes from healthy subjects but not patients with AIDS. Lower doses of IFN-γ (200 U/ml) were used in those studies. In dose-response studies, however, we observed comparable levels of intracellular *M. avium* growth inhibition in monocytes from patients with AIDS and healthy subjects at doses of IFN-γ ranging from 10 to 300 U/ml. Other cytokines, including interleukins 2, 3, 4, and 6 and macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor, failed to augment intracellular *M. avium* growth inhibition in monocytes from healthy donors (35). Only IFN-γ further enhanced intracellular *M. avium* killing.

Murray et al. have demonstrated that mononuclear cells from patients with AIDS produce subnormal levels of IFN-γ

in response to nonspecific mitogenic stimuli (19) and when stimulated by heat-killed BCG or soluble *M. intracellulare* antigens (20). The surface expression of receptors for IFN-γ on peripheral blood mononuclear cells from patients with AIDS and AIDS-related complex was normal (16). When stimulated in vitro by exogenous IFN-γ, monocytes from patients with AIDS showed increased oxidative-burst activity and antimicrobial activity against *T. gondii* and *C. psittaci* comparable to those of IFN-γ-stimulated monocytes from healthy subjects (19). Our data extend these observations by demonstrating that continuous culture of monocytes from patients with AIDS in the presence of IFN-γ results in augmentation of the ability of these cells to inhibit intracellular *M. avium* growth. Whereas the observed in vitro effects of IFN-γ are small, the culture condition may introduce a bias in favor of the bacteria, and options for treatment of disseminated *M. avium* infections remain extremely limited. Limited clinical trials of recombinant human IFN-γ in combination with antimycobacterial drug therapy in patients with AIDS and disseminated *M. avium* infection are ongoing (39). Given the complexity of the host-microbial interactions in *M. avium* infection and our present inability to reconstitute or restore $CD4^+$ lymphocyte function in patients with AIDS, multiple modality therapy with both immunomodulating substances and antimycobacterial drug therapy, therefore, may offer a reasonable therapeutic option.

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