INFECTION AND IMMUNITY, Sept. 1988, p. 2250–2254
0019-9567/88/092250-05$02.00/0

Vol. 56, No. 9

Growth of *Mycobacterium avium* in Activated Macrophages Harvested from Inbred Mice with Differing Innate Susceptibilities to Mycobacterial Infection

R. W. STOKES AND F. M. COLLINS*

Trudeau Institute, Inc., Saranac Lake, New York 12983

Received 31 March 1988/Accepted 3 June 1988

The growth of *Mycobacterium avium* in macrophages obtained from *Mycobacterium bovis* BCG-infected mice was compared with that in macrophages from uninfected mice. BCG vaccination resulted in substantial macrophage activation, measured as increased acid phosphatase and superoxide anion production, as well as enhanced leishmanicidal activity. However, the activated macrophages were only able to reduce the rate of intracellular growth by *Listeria monocytogenes* and *M. avium* in vivo and did not express detectable levels of mycobactericidal activity in vitro. Exposure of the macrophage monolayers to concanavalin A-stimulated spleen cell supernatant fluid and lipopolysaccharide did not further enhance the ability of the BCG-activated macrophages to control the intracellular replication of the *M. avium*. Macrophages from BCG-infected C57BL/6 (BCG^s) mice were quantitatively better able to control the intracellular replication of the *M. avium* challenge than were similar phagocytes obtained from BCG^r (A/J) mice. These findings have important implications with respect to the expression of acquired resistance to these atypical mycobacterial infections.

Many nontuberculous mycobacteria are opportunistic human pathogens, capable of causing disease in normal adults only when there is some other underlying disease which potentiates the severity of the mycobacterial infection (23). The prevalence of chronic lung infections (caused by *Mycobacterium kansasii* and members of the *Mycobacterium avium* complex) has not declined within the United States in a manner comparable to that of *Mycobacterium tuberculosis* lung infections (13). Although these organisms are less virulent than *M. tuberculosis*, they can still persist within the lung for long periods of time (4, 5). One explanation for this persistence is their ability to resist the microbicidal capacity of activated macrophages, which are generated as a result of the cell-mediated immune response (14, 15). A number of studies have been made of the interactions occurring between the tubercle bacillus and these activated macrophages (6, 9, 17), but there is generally much less known about the response by the atypical mycobacteria to these cells, especially when tested in vitro (8).

Earlier studies have indicated substantial differences between the growth rates for *M. avium* when introduced into normal macrophages which have been harvested from naturally susceptible C57BL/6 and resistant A/J inbred strains of mice (21). The present study shows that immunologically activated macrophages taken from these same mice are incapable of killing the *M. avium*, although they did reduce their rate of intracellular replication to some extent.

MATERIALS AND METHODS

Bacteria. *M. avium* (TMC 702) and *Mycobacterium bovis* (BCG Pasteur, TMC 1011) were obtained from the Trudeau Mycobacterial Culture Collection, Saranac Lake, N.Y. They were grown in modified Sauton medium with added Tween 80 as described previously and stored in 1-ml ampoules at −70°C until required (3). The frozen suspension was thawed and briefly sonicated to break up any clumps before being diluted to the desired concentration in 0.05% Tween 80–saline. The number of viable bacteria in each preparation was checked by plating serial dilutions on Middlebrook 7H11 agar (Difco Laboratories, Detroit, Mich.) and counting the resultant colonies after 21 days of incubation in sealed plastic bags at 37°C.

Listeria monocytogenes EGD (serotype 1/2a) was grown in tryptic soy broth (Difco), harvested in the midlogarithmic growth phase, and stored in 1-ml samples at −70°C until required. The intravenous 50% lethal dose was approximately 10^5 bacteria for the AB6 mice, determined after 10 days by the Reed Muensch method (3).

Mice. Specific-pathogen-free C57BL/6 (naturally susceptible) and A/J and AB6 (A × C57BL/6)F_1 male (naturally resistant) mice between 6 and 10 weeks old were obtained from the Trudeau Institute Animal Breeding Facility, Saranac Lake, N.Y. They were raised under barrier conditions and fed sterile mouse chow and acidified water ad libitum (15).

Demonstration of nonspecific resistance in BCG-infected mice. A/J and C57BL/6 mice were infected with 10^6 CFU of BCG suspended in 0.2 ml of phosphate-buffered saline, which was injected via a lateral tail vein (3). Twenty-one days later, the vaccinated mice (along with naive, age-matched controls) were challenged intravenously with either *M. avium* (10^4 or 10^7 CFU per mouse) or *L. monocytogenes* (10^5 CFU per mouse) in 0.2 ml of phosphate-buffered saline. The subsequent growth of the challenge infection within the spleen and liver was followed by plating 10-fold dilutions of the organ homogenates on either tryptic soy agar (*L. monocytogenes*) or on Middlebrook 7H11 agar (*M. avium*), the latter being supplemented with 1 μg of 2-thiophenecarboxylic acid hydrazide per ml of 7H11 agar to inhibit BCG growth (3). Bacterial colonies were enumerated after incubation at 37°C in a humidified atmosphere for 21 days for *M. avium* and 24 h for *L. monocytogenes*.

Infection of macrophages in vitro. Monolayers of peritoneal macrophages were prepared from mice infected with 10^6 CFU of BCG injected intravenously 21 days earlier and elicited by an intraperitoneal injection (3 days before collect-

* Corresponding author.

ing the washouts) of 2 ml of sterile 3.8% casein (Eastman Kodak Co., Rochester, N.Y.) plus 10^7 heat-killed BCG organisms per ml. The peritoneal exudate cells were suspended at a concentration of 5×10^5 cells per ml in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) containing 20% fetal calf serum (GIBCO), 1 mM sodium pyruvate, 2 mM L-glutamine, and 10 mM *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer solution (Whittacker Bioproducts, Walkerville, Md.). Samples (1 ml) of the cell suspension were added to replicate cover slips in 24-well cluster plates and incubated for 2 h at 37°C in 6% CO_2-enriched air. Nonadherent cells were removed by washing with PBS, and 1 ml of fresh complete medium containing 10^6 CFU of mycobacteria was then added. After 4 h incubation at 37°C, the uningested mycobacteria were removed by vigorously washing the cover slips with phosphate-buffered saline before transferring them to fresh medium in new 24-well plates. At this time, and again after 7 days of incubation at 37°C in 6% CO_2-enriched air, three cover slips were processed to determine the number of viable intracellular bacteria per 10^5 adherent macrophages. To each cover slip, 0.25 ml of 0.05% napthol blue black in 0.1 M citric acid plus 1% Triton X-100 (12) was added; after agitation, a count was made of the blue-stained macrophage nuclei. In addition, 0.1 ml of the lysate was removed from each well and transferred into 0.9 ml of 0.1 M Na_2HPO_4, briefly sonicated to break up any clumps of bacteria, and plated on Middlebrook 7H11 agar to determine the number of viable *M. avium*. The counts are expressed as the number of viable bacteria per 10^5 adherent macrophages. The number of times the intracellular mycobacteria had doubled in number over the 7 day incubation period was calculated as follows: replication index = Log_2 [(CFU/10^5 macrophages at 7 days)/(CFU/10^5 macrophages at 4 h].

Demonstration of macrophage activation in vitro. Three parameters of macrophage activation were determined for the macrophages used in the present experiments. Macrophage monolayers prepared from both normal and BCG-infected mice were assayed simultaneously for acid phosphatase activity, superoxide anion production, and leishmanicidal capacity.

Acid phosphatase activity. Monolayers were prepared in 75-cm^2 tissue culture flasks (Becton Dickinson Labware, Oxnard, Calif.). After 4 h of incubation at 37°C in 6% CO_2-enriched air, nonadherent cells were removed by three washes with sterile phosphate-buffered saline. Distilled water (3 ml) containing 1% Triton X-100 was then added to each flask, and flasks were frozen and thawed three times. The resultant cell lysates were removed and assayed for acid phosphatase activity by using *p*-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, Mo.) as the substrate (2). Results are expressed as nanomoles of *p*-nitrophenol produced per milligram of protein per 30-min period. Protein was assayed by using the bicinchoninic acid assay against a bovine serum albumin standard curve (19).

Superoxide anion production. Monolayers were prepared in 24-well cluster plates without cover slips. After 4 h of incubation at 37°C to allow adherence, the monolayers were washed, and 1 ml of complete medium containing 1 mg of nitroblue tetrazolium (Sigma) per ml was added to each well. Phorbolmyristate acetate (Sigma) was added at a final concentration of 50 ng/ml.

Monolayers were incubated for 60 min at 37°C in 6% CO_2-enriched air. For each experimental group, three wells were processed to assess the average number of adherent cells in the monolayer, and three other wells were processed to assess the amount of reduced nitroblue tetrazolium present (18). Superoxide anion production was expressed as nanomoles of nitroblue tetrazolium reduced per 10^6 macrophages per 60 min.

Leischmanicidal assay. Macrophage monolayers were prepared on cover slips in 24-well cluster plates. After 2 h of incubation at 37°C, the monolayers were washed to remove nonadherent cells, and 1 ml of complete medium containing 5×10^5 *Leishmania mexicana amazonensis* (strain Maria) promastigotes (kindly provided by J. O. Hill, Trudeau Institute) was added to each well. After 4 h of incubation at 32°C, uningested parasites were removed by washing, and fresh complete medium was added. At this time, and again after 3 days of infection, three replicate cover slips were fixed and stained with 6% Giemsa (Polysciences, Inc., Warrington, Pa.). The number of intracellular amastigotes within 100 macrophages was assessed microscopically for each monolayer and the percentage difference between amastigote numbers at 4 h and 3 days was calculated.

Lymphokine production. Normal AB6 F_1 mice were infected intravenously with 10^6 CFU of BCG. After 21 days the spleens were removed and dissociated by passage through a stainless steel sieve into RPMI medium plus 5% fetal calf serum. Erythrocytes were lysed with buffered ammonium chloride (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA), and clumps of cells were removed by sedimentation. The resulting single-cell suspension was diluted in RPMI medium plus 5% fetal calf serum to a concentration of 2×10^6 cells per ml. The cell suspension (50 ml) was dispensed into 75-cm^2 tissue flasks, and concanavalin A (Sigma) was added to give a final concentration of 2 μg/ml. The cultures were incubated at 37°C in 6% CO_2-enriched air for 3 days, the cells were removed by centrifugation (150 × *g* for 15 min), and the supernatant fluid was sterilized by filtration. Stimulated spleen cell supernatants (lymphokine) were stored at −20°C until required.

Statistics. Collected data were analyzed by using analysis of variance and the Student *t* test for a difference between two of several independent means with the Statview 512+ program (Brainpower Inc., Calabasas, Calif.) run on a MAC plus microcomputer.

RESULTS

Growth of *M. avium* in normal and BCG-infected mice. A/J and C57BL/6 mice were infected with about 10^6 CFU of strain BCG Pasteur and 21 days later were challenged (along with age-matched, uninfected mice) with either 10^4 or 10^7 CFU of *M. avium*. Growth of the *M. avium* challenge infection in the spleen and liver is shown in Fig. 1. The BCG-vaccinated C57BL/6 mice limited the growth of the *M. avium* challenge more effectively than did the BCG-infected A/J mice. As a result, 10 days after administering the low dose, 10^4 CFU of *M. avium* challenge, a significant reduction in the bacterial load was seen in both the spleens and livers of the BCG-vaccinated C57BL/6 mice ($P < 0.05$). Furthermore, this reduction continued throughout the experiment, the numbers within the BCG-vaccinated C57BL/6 mice remaining 100- to 1,000-fold lower than those in the normal controls (Fig. 1A). On the other hand, the BCG-infected A/J mice were relatively much less protected against the subsequent challenge, at least judging from the bacterial load seen in the livers of the normal mice compared with those in the BCG-vaccinated A/J mice on day 60. Some protection ($P < 0.05$) was observed in the spleen 20 days into the low *M. avium* challenge infection (Fig. 1A), but both groups of

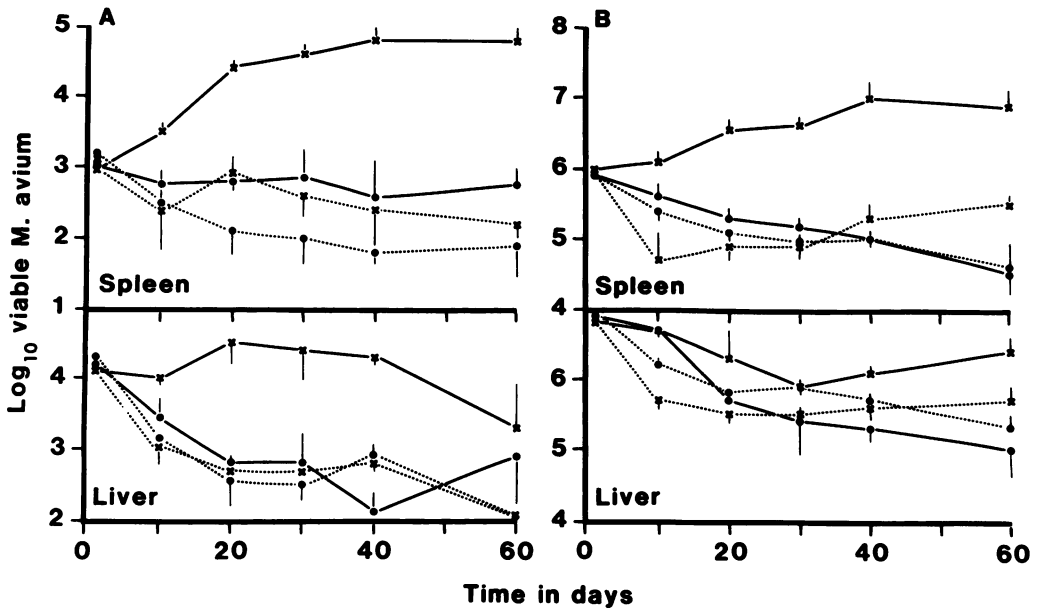

FIG. 1. Growth of 10^4 (A) or 10^7 CFU (B) of *M. avium* in C57BL/6 (×) or A/J (●) mice. Mice had either been infected with 10^6 CFU of BCG Pasteur 21 days earlier (---) or were age-matched untreated control mice (——). Vertical bars represent the standard errors of the means for five replicate determinations.

vaccinated mice receiving the larger (10^7 CFU) *M. avium* challenge exhibited similar viable counts (Fig. 1B); the difference in the A/J mice was not sufficient to be significant. On the other hand, the heavily challenged C57BL/6 mice exhibited significant differences ($P < 0.05$) from day 10 of the challenge.

Growth of *L. monocytogenes* in normal and BCG-vaccinated mice. The above experiment suggested that BCG-infected A/J mice did not express effective levels of acquired resistance against the subsequent *M. avium* challenges. However, the innately resistant host developed some activated macrophages in response to the BCG infection, since the BCG-infected A/J mice challenged intravenously with a 5 times the 50% lethal dose of *L. monocytogenes* showed a substantial reduction in the growth of the *L. monocytogenes* in both the liver and spleen over a 7-day period (Fig. 2). In the normal control mice, the *L. monocytogenes* multiplied progressively in vivo, resulting in the death of all of the A/J mice. On the other hand, the C57BL/6 mice readily limited this challenge to sublethal proportions so that most of the animals survived. BCG vaccination afforded increased anti-*L. monocytogenes* protection to both the A/J and the C57BL/6 mice, resulting in a 1,000-fold reduction in the bacterial load in both the liver and spleen, thereby protecting all of the A/J mice from death.

Quantitative estimates of macrophage activation in vitro. Resistance to a *Listeria* challenge was one measure of nonspecific macrophage activation (14). Other parameters of macrophage activation, such as acid phosphatase activity, were also increased substantially (approximately twofold) in BCG-infected A/J and C57BL/6 mouse macrophages compared with that observed in normal controls (Table 1). On the other hand, superoxide anion production (in response to phorbolmyristate acetate stimulation) was enhanced in the C57BL/6 macrophages but not in cells harvested from BCG-infected A/J mice. Such activated macrophages were able to kill leishmanias; the C57BL/6 cells were more active in this regard (Table 1). Macrophages harvested from normal A/J and C57BL/6 mice were consistently unable to kill leishmanias.

Growth of mycobacteria in macrophage monolayers prepared from normal and BCG-infected mice. Having established that macrophages obtained from A/J and C57BL/6

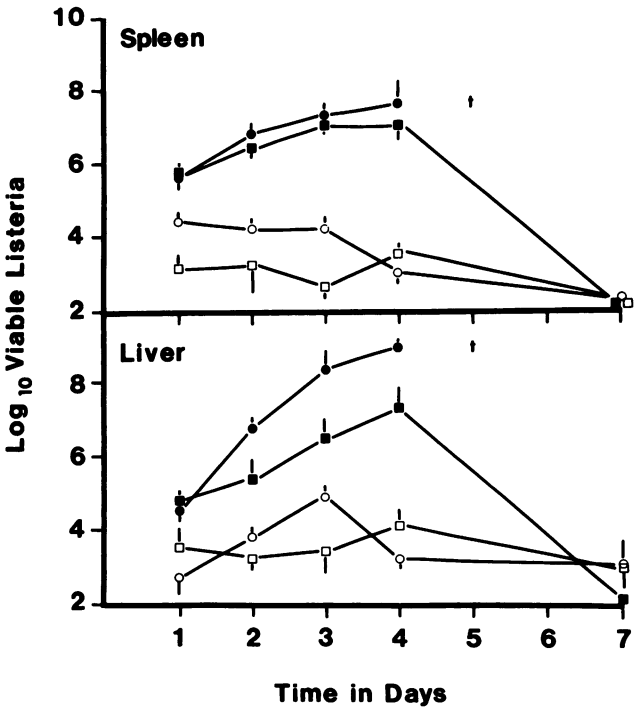

FIG. 2. Growth of 10^5 viable *L. monocytogenes* in C57BL/6 (■, □) or A/J (●, ○) mice. Mice had been infected with 10^6 CFU of BCG Pasteur 21 days before the *Listeria* challenge (□, ○) or were age-matched untreated mice (■, ●). Vertical bars represent the standard errors of the means for four determinations.

TABLE 1. Parameters of activation in peritoneal macrophages obtained from normal and BCG infected C57BL/6 and A/J mice

Macrophage source[a]	Acid phosphatase activity[b]	O_2^- production[c]	Growth of *Leishmania*[d] (%)
A/J control	51.4 ± 10.7[5]	28.4 ± 1.9[5]	+5
A/J BCG	97.6 ± 11.1[e]	39.0 ± 8.3[f]	−37
C57BL/6 control	44.1 ± 8.1	36.0 ± 8.7	+59
C57BL/6 BCG	87.6 ± 6.3[g]	92.7 ± 3.1[h]	−76

[a] Elicited peritoneal macrophages were obtained from uninfected mice (control) or mice infected with 10^6 CFU of BCG 21 days previously (BCG).
[b] Nanomoles of *p*-nitrophenol produced per milligram of protein per 30 min (mean ± standard error; $n = 3$).
[c] Nanomoles of nitroblue tetrazolium reduced per 10^6 macrophages per 60 min (mean ± standard error; $n = 3$).
[d] Percent difference in intracellular amastigote numbers after a 3-day incubation period.
[e] $P < 0.05$ when compared with corresponding normal group.
[f] $P > 0.05$ when compared with corresponding normal group.
[g] $P < 0.01$ when compared with corresponding normal group.
[h] $P < 0.001$ when compared with corresponding normal group.

mice were both activated to some degree by BCG vaccination, we next attempted to quantitate their mycobactericidal capacity. Growth of *M. avium* in both groups of BCG-activated macrophages is shown in Fig. 3, together with the corresponding curves for normal control cells. The rate of growth shown by the *M. avium* when taken up by normal C57BL/6 (naturally susceptible) macrophages was consistently greater than that seen in the A/J (naturally resistance) cells. However, both groups of macrophages harvested from BCG-infected mice showed reduced growth by the intracellular mycobacteria during the 7-day incubation period. The extent of this reduction was greater in the BCG-activated C57BL/6 macrophages, resulting in a significant difference ($P < 0.05$) between the *M. avium* counts in the control versus BCG-activated macrophage monolayers. Similar results were obtained when normal and BCG-activated macrophages were challenged with the homologous strain (*M. bovis* BCG) of mycobacteria (Fig. 4).

Effect of lymphokine and lipopolysaccharide stimulation on the ability of the macrophage to limit the growth of *M. avium*. Macrophages seem to require two signals to become fully activated (10). This can be achieved by adding a lymphokine, followed by trace amounts of lipopolysaccharide. The growth of *M. avium* within macrophages which have received this multiple signal is shown in Fig. 5, which indicates

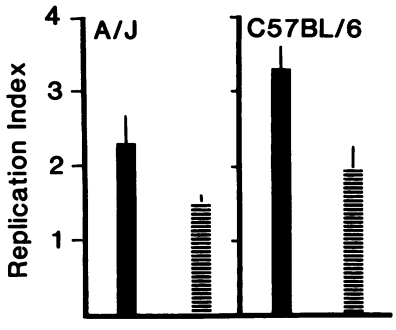

FIG. 3. Replication of *M. avium* in elicited macrophages obtained from normal (■) or BCG-infected (▤) C57BL/6 and A/J mice. See Materials and Methods for an explanation of the replication index. Vertical bars represent the standard errors of the means for seven separate experiments.

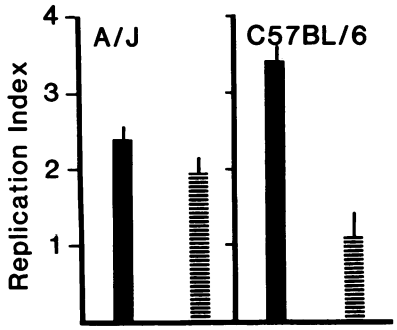

FIG. 4. Replication of BCG Pasteur in elicited macrophages obtained from normal (■) or BCG-infected (▤) C57BL/6 and A/J mice. Vertical bars represent standard errors for five experiments.

that the intracellular replication was significantly retarded by the C57BL/6 macrophages ($P < 0.05$) but not by those from the A/J mice ($P > 0.05$) when compared with that observed for normal cells. The separate addition of 50% lymphokine or lipopolysaccharide to normal or activated macrophages showed no evidence of bacteriostasis; in fact, these control treatments seemed to enhance the growth of the *M. avium* to some extent, although the counts were not statistically significantly different from those for the unstimulated macrophages ($P > 0.05$).

DISCUSSION

The present study confirms the fact that macrophages harvested from infected mice are substantially activated, both in terms of acid phosphatase and superoxide anion production, as well as inactivation of *Listeria* and *Leishmania* species (7). However, these same activated macrophages seem incapable of killing a standardized challenge inoculum of *M. avium* (or even BCG), although they could reduce their rates of intracellular growth substantially (Fig. 3 and 4). The BCG-activated macrophages taken from the susceptible C57BL/6 mice were quantitatively more effective at slowing the intracellular growth of these mycobacteria, compared with cells harvested from the naturally resistant mice (A/J). This difference correlated well with enhanced superoxide anion production after phorbolmyristate acetate stimulation

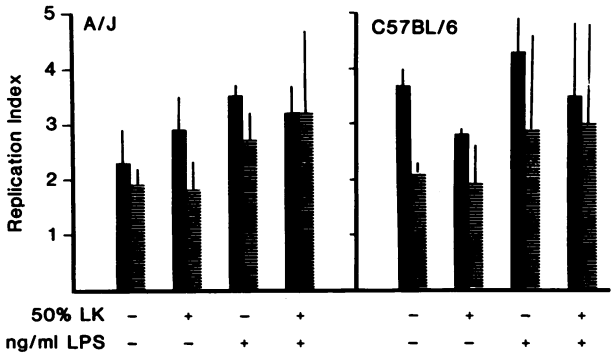

FIG. 5. Replication of *M. avium* in elicited macrophages obtained from normal (■) or BCG-infected (▤) C57BL/6 and A/J mice. Lipopolysaccharide (LPS; 50 ng/ml, final concentration) or lymphokine (50%, final concentration) were (+) or were not (−) added to macrophages. Vertical bars represent the standard errors of the means for three separate experiments.

as well as a greater degree of leischmanicidal activity. On the other hand, acid phosphatase levels in BCG-activated macrophages from either C57BL/6 or A/J mice were comparable in activity, emphasizing the divergence in functional capacities exhibited by macrophages activated with live BCG vaccine (22). However, the present studies generally support the contention that activated macrophages produced by resistant strains of mice are functionally defective, at least in some activities (11).

The demonstration that BCG-activated macrophages can restrict the growth of *M. avium* in an in vitro test environment extends earlier reports of such activity in other mycobacteira (1, 6, 9, 17). However, lymphokine-activated macrophages showed no demonstrable bacterial activity in vitro, a finding in sharp contrast to earlier reports with *M. tuberculosis* (6, 20). This difference may simply reflect the experimental models used in these tests, or it may relate to the greater resistance shown by *M. avium* to inactivation by activated macrophages in general (14).

Lipopolysaccharide added to these cells in trace quantities is said to provide a second signal which transforms the primed macrophage into one with bactericidal qualities (10). Thus, the mycobactericidal (or at least mycobacteriostatic) activity of the BCG-activated macrophages could be enhanced by prior lipopolysaccharide treatment of the cells. The results obtained in the present study do not support this lipopolysaccharide trigger hypothesis since, if anything, lipopolysaccharide treatment stimulated mycobacterial growth within the BCG-activated macrophages (Fig. 5), suggesting that some sort of antagonistic action may occur after lipopolysaccharide treatment of the BCG-stimulated macrophage (16).

The best that the BCG-activated macrophage could achieve was the inhibition of growth by the *M. avium* challenge within the phagocyte. This relatively slight reduction in growth by the *M. avium* (Fig. 3) contrasted with the active killing of a secondary *M. avium* challenge population in vivo (Fig. 1). Inefficiency of growth reduction by the BCG-activated macrophage seems dependent upon the genetic makeup of the host, so that activated macrophages harvested from the naturally resistant A/J mice failed to inhibit the growth of the *M. avium* challenge substantially, whereas similar phagocytes obtained from the naturally susceptible mice (C57BL/6) could restrict the growth of the *M. avium* population to some degree. The reason for this apparent paradox remains unclear and deserves further examination.

ACKNOWLEDGMENTS

We thank L. Auclair, M. Lincoln, and C. Niederbuhl for their excellent technical assistance.

This work was supported by Public Health Service grants HL-19774 and AI-14065 from the National Institutes of Health and by a grant from the New York AIDS Institute, Albany, N.Y.

LITERATURE CITED

1. **Alexander, J., and C. C. Smith.** 1978. Growth of *Mycobacterium lepraemurium* in nonstimulated and stimulated mouse peritoneal-derived and bone marrow-derived macrophages in vitro. Infect. Immun. **22**:631–636.
2. **Barrett, A. J., and M. F. Heath.** 1977. Lysosomal enzymes, p. 19–105. *In* J. T. Dingle (ed.), Lysosomes: a laboratory handbook, 2nd ed. Elsevier Biomedical Press, Amsterdam.
3. **Collins, F. M., and G. B. Mackaness.** 1970. The relationship of delayed hypersensitivity to acquired antituberculous immunity. I. Tuberculin sensitivity and resistance to reinfection in BCG-vaccinated mice. Cell. Immunol. **1**:253–265.
4. **Collins, F. M., N. E. Morrison, and V. Montalbine.** 1978. Immune response to persistent mycobacterial infection in mice. Infect. Immun. **20**:430–438.
5. **Collins, F. M., and S. R. Watson.** 1981. Immune response to atypical mycobacterial lung infections. Rev. Infect. Dis. **3**:981–989.
6. **Crowle, A. J., and M. May.** 1981. Preliminary demonstration of human tuberculoimmunity in vitro. Infect. Immun. **31**:453–464.
7. **Czuprynski, C. J., P. M. Henson, and P. A. Campbell.** 1984. Killing of *Listeria monocytogenes* by inflammatory neutrophils and mononuclear phagocytes from immune and nonimmune mice. J. Leuk. Biol. **35**:193–208.
8. **Gangadharam, P. R. J., and P. F. Pratt.** 1983. *In vitro* response of murine alveolar and peritoneal macrophages to *Mycobacterium intracellulare*. Am. Rev. Respir. Dis. **128**:1044–1047.
9. **Godal, R., R. J. W. Rees, and J. O. Lamvik.** 1971. Lymphocyte-mediated modification of blood-derived macrophage function *in vitro*-inhibition of growth of intracellular mycobacteria with lymphokines. Clin. Exp. Immunol. **8**:625–637.
10. **Hamilton, T. A., and D. O. Adams.** 1987. Molecular mechanisms of signal transduction in macrophages. Immunol. Today **8**:151–158.
11. **James, S. L., E. Skamene, and M. S. Meltzer.** 1983. Macrophages as effector cells of protective immunity in murine schistosomiasis. V. Variation in macrophage schistosomulocidal and tumoricidal activities among mouse strains and correlation with resistance to reinfection. J. Immunol. **131**:948–953.
12. **Nakagawora, A., and C. F. Nathan.** 1983. A simple method for counting adherent cells: application to cultured human monocytes, macrophages and multinucleated giant cells. J. Immunol. Methods **56**:261–268.
13. **O'Brien, R. J., L. J. Geiter, and D. E. Snider, Jr.** 1987. The epidemiology of nontuberculous mycobacterial diseases in the United States. Results from a National Survey. Am. Rev. Respir. Dis. **135**:1007–1014.
14. **Orme, I. M., and F. M. Collins.** 1983. Resistance of various strains of mycobacteria to killing by activated macrophages *in vivo*. J. Immunol. **131**:1452–1454.
15. **Orme, I. M., R. W. Stokes, and F. M. Collins.** 1986. Genetic control of natural resistance to nontuberculous mycobacterial infection in mice. Infect. Immun. **54**:56–62.
16. **Peavy, D. L., R. E. Baughn, and D. M. Musher.** 1979. Effects of BCG infection on the susceptibility of mouse macrophages to endotoxin. Infect. Immun. **24**:59–64.
17. **Rook, G. A. W., J. Steele, M. Ainsworth, and B. R. Champion.** 1986. Activation of macrophages to inhibit proliferation of *Mycobacterium tuberculosis*: comparison of the effects of recombinant gamma-interferon on human monocytes and murine peritoneal macrophages. Immunology **59**:333–338.
18. **Rook, G. A. W., J. Steele, S. Umar, and H. M. Dockrell.** 1985. A simple method for the solubilization of reduced NBT and its use as a colorimetric assay for activation of human macrophages by gamma-interferon. J. Immunol. Methods **82**:161–167.
19. **Smith, P. K., R. I. Krohn, G. T. Mermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujinioko, N. M. Goethe, B. J. Olson, and D. C. Klenk.** 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. **150**:76–85.
20. **Steele, J., K. C. Flint, A. L. Pozniak, B. Hudspith, M. M. Johnson, and G. A. W. Rook.** 1986. Inhibition of virulent *Mycobacterium tuberculosis* by murine peritoneal macrophages and human alveolar lavage cells: the effects of lymphokines and recombinant gamma-interferon. Tubercle **67**:289–294.
21. **Stokes, R. W., I. M. Orme, and F. M. Collins.** 1986. Role of mononuclear phagocytes in expression of resistance and susceptibility to *Mycobacterium avium* infections in mice. Infect. Immun. **54**:811–819.
22. **VanDissel, J. T., J. J. M. Stikkelbroek, M. T. van den Barselaar, W. Sluiter, P. C. J. Leigh, and R. van Furth.** 1987. Divergent changes in antimicrobial activity after immunologic activation of mouse peritoneal macrophages. J. Immunol. **139**:1665–1677.
23. **Wolinsky, E.** 1979. Nontuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. **119**:107–159.