

Enhancement of Growth of *Mycobacterium lepraemurium* in Macrophages by Gamma Interferon

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Gamma interferon, an immune lymphokine that protects mouse macrophages against infection by several parasites, was ineffective against *Mycobacterium lepraemurium*. On the contrary, it significantly stimulated multiplication of *M. lepraemurium* in the macrophages. Simultaneous treatment of macrophages with gamma interferon and interleukin-4 or interleukin-2 or a combination of all three did not enhance the macrophage resistance to infection with *M. lepraemurium*, but instead stimulated growth of *M. lepraemurium*.

Mycobacterium lepraemurium is an intracellular rodent pathogen which multiplies in macrophages. Injected intravenously in mice, it causes a slowly progressive, ultimately fatal systemic infection. It multiplies copiously in the mouse, which in late stages of the disease harbors enormous bacterial populations. The reason for this profuse multiplication is unknown; however, it does seem to be associated with profound suppression of cellular immunity (8).

Lymphokines and especially gamma interferon (IFN- γ) are instruments of cell-mediated immunity, generally activating macrophages to resist or kill various intracellular pathogens (7). Their roles in antimycobacterial immunity are somewhat confusing. Crude lymphokine-activated mouse macrophages have been reported to kill *Mycobacterium microti* (10). Recombinant IFN- γ (rIFN- γ) appears to protect mouse macrophages against *M. tuberculosis* (5, 9) but does not protect human macrophages, in which it may actually have an anti-immune effect and enhance bacterial growth (3, 4, 9). Bovine alpha IFN has been reported to protect bovine monocytes but not monocyte-derived macrophages against *Mycobacterium paratuberculosis*, a close relative of *M. lepraemurium* (12). Crude lymphokines were found to activate peritoneal mouse macrophages to stop *M. lepraemurium* replication and to activate bone marrow macrophages to kill *M. lepraemurium* (1). Here we report evidence that mouse rIFN- γ can significantly enhance *M. lepraemurium* multiplication in cultured mouse peritoneal macrophages. The combination of IFN with either recombinant interleukin 2 (IL-2) or recombinant interleukin-4 (IL-4) or both had no effect on macrophage resistance to *M. lepraemurium* infection.

Resident peritoneal macrophages obtained from murine pathogen-free Swiss Webster mice (Taconic) by saline lavage were cultured on glass cover slips in glass Leighton tubes in Dulbecco modified Eagle medium (6) supplemented with 10% heat-inactivated fetal calf serum and lymphokines (rIFN- γ , obtained from Genentech, was provided by P. A. Campbell, National Jewish Center for Immunology and Respiratory Medicine in the framework of our ongoing collaborative studies; recombinant IL-2 and recombinant IL-4 were purchased from the Genzyme Corporation, Boston, Mass.). The medium was replaced every 3 to 4 days, and the lymphokines were continuously present during the

infection. The macrophages were infected with *M. lepraemurium* harvested from the livers of *M. lepraemurium*-infected BALB/c mice and purified by centrifugation on Percoll gradients (11). A one-use portion from a standard suspension of *M. lepraemurium* stored at -80°C was used at 2×10^7 acid-fast bacilli per ml in Hanks balanced salt solution without opsonization, for a proportion of bacteria to cultured macrophages of approximately 20:1. The infection was for 50 min at 37°C in 5% CO_2 and 95% air. Unphagocytized bacteria were washed off with saline. The infected macrophages were incubated for the periods indicated. Representative cultures were fixed in methanol and stained with carbol fuchsin, and the number of acid-fast bacilli per macrophage was determined from counting at least 200 macrophages on each cover slip. The results were expressed as mean acid-fast bacilli per macrophage \pm standard deviation for three observations.

Macrophages incubated with IFN- γ or IL-4 or both exhibited an increase in size and spreading on surfaces compared with control or IL-2-incubated macrophages. IFN- γ -treated macrophages showed a slightly lower rate of phagocytosis than did the control macrophages, but in general, addition of the lymphokines did not have a significant effect on the percentage of macrophages infected with *M. lepraemurium*. The numbers of *M. lepraemurium* in control macrophages increased approximately six times in the first 12 days of culture but did not change much in an additional 2 weeks of incubation (see medium control group, Fig. 1). This shows that rIFN- γ in the medium markedly stimulated *M. lepraemurium* replication in the macrophages. They increased approximately 18-, 24-, and 26-fold, depending on the dose of interferon. Higher concentrations (5,000 and 10,000 U/ml) were used in additional experiments. While these also tended to stimulate intracellular *M. lepraemurium* multiplication, they were not well tolerated by the macrophages; a concentration of 10,000 U/ml exerted a toxic effect on macrophages.

Recent studies have shown that some macrophage antimicrobial activities can be induced by two or three lymphokines (2). To test this possibility in our system, macrophage monolayers were incubated with IL-4 alone or simultaneously with IFN and IL-4 for 24 h and then infected with *M. lepraemurium*. Under neither circumstance were macrophages stimulated to resist *M. lepraemurium* multiplication. Still, when IFN and IL-4 were used in combination, a slight antagonism of the IFN effect was suggested (Fig. 2). Subse-

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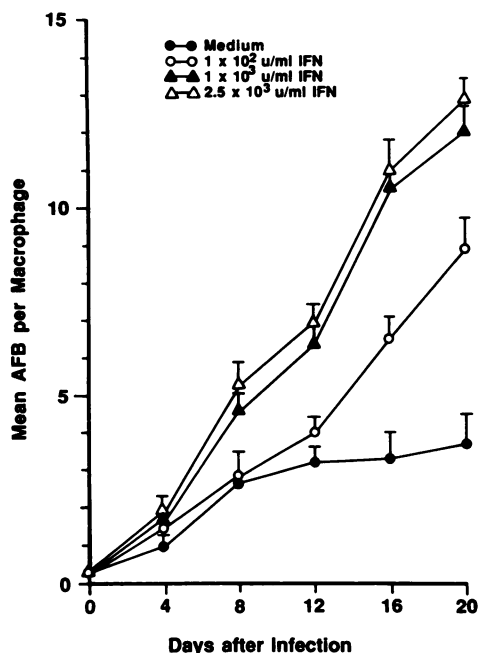


FIG. 1. rIFN- γ concentration-related enhancement of *M. lepraemurium* multiplication in cultured mouse peritoneal macrophages. IFN was in the culture medium at the concentrations indicated from 24 h before infection throughout the length of incubation.

quent experiments showed that a combination of IFN with IL-2 or with both IL-4 and IL-2 was ineffective (data not shown). In short, in every instance that IFN was present in the medium, a higher multiplication of *M. lepraemurium* was noticed than in its absence. In some experiments, infected macrophages were lysed with distilled water or by sonication and were incubated with IFN in their medium. No increase in the number of *M. lepraemurium* was found during 3 months. This shows that the macrophages are necessary for enhanced multiplication of *M. lepraemurium*.

These results show that mouse rIFN- γ supports and promotes multiplication of *M. lepraemurium* within mouse

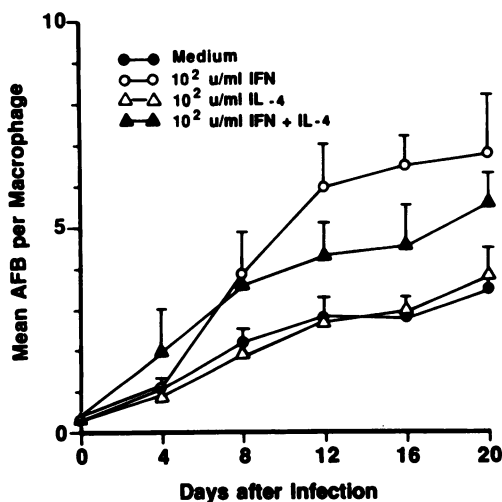


FIG. 2. The effect of IFN- γ , IL-4, or IFN plus IL-4 for induction of macrophage resistance to *M. lepraemurium* infection. Lymphokines (100 U/ml) were in the culture medium from 24 h before infection throughout the length of incubation.

macrophages; the data would appear to provide direct evidence that IFN- γ may play a pathogenetic role in *M. lepraemurium* infection. As one interpretation for higher multiplication of bacilli, we observed that under the influence of IFN the macrophages appeared to be noticeably stimulated, suggesting that they probably pinocytose almost voraciously in this medium. The endocytosed nutrients are delivered via the lysosomal compartment to phagosomes enclosing the *M. lepraemurium*, since it is well known that this organism resides (and presumably thrives) in mouse macrophage phagolysosomes. It seems reasonable, therefore, that under the influence of IFN- γ the phagolysosome environment may provide even an overplus of nutrients from the medium to the entrapped microorganism and that this strongly promotes growth.

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LITERATURE CITED

- 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.
- Belosevic, M., C. E. Davis, M. S. Meltzer, and C. A. Nacy. 1988. Regulation of activated macrophage antimicrobial activities. Identification of lymphokines that cooperate with IFN-gamma for induction of resistance to infection. *J. Immunol.* **141**:890-896.
- Crowle, A. J. 1988. The tubercle bacillus—human macrophage relationship studied *in vitro*, p. 99-136. In H. Friedman and M. Bendinelli (ed.), *Mycobacterium tuberculosis*. Plenum Publishing Corp., New York.
- Douvas, G. S., D. L. Looker, A. E. Vatter, and A. J. Crowle. 1985. Gamma interferon activates human macrophages to become tumoricidal and leishmanicidal but enhance replication of macrophage-associated mycobacteria. *Infect. Immun.* **50**:1-8.
- Flesch, I. E. A., and S. H. E. Kaufmann. 1988. Attempt to characterize the mechanisms involved in mycobacterial growth inhibition by gamma-interferon-activated bone marrow macrophages. *Infect. Immun.* **56**:1464-1469.
- Mor, N., and M. B. Goren. 1987. Discrepancy in assessment of phagosome-lysosome fusion with two lysosomal markers in murine macrophages infected with *Candida albicans*. *Infect. Immun.* **55**:1663-1667.
- Murray, H. W. 1988. Interferon-gamma, the activated macrophage and host defense against microbial challenge. *Ann. Intern. Med.* **108**:595-608.
- Ptak, W., J. M. Gaugas, R. J. W. Rees, and A. C. Allison. 1970. Immune responses in mice with murine leprosy. *Clin. Exp. Immunol.* **6**:117.
- 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 effect of recombinant gamma-interferon on human monocytes and murine peritoneal macrophages. *Immunology* **59**:333-338.
- Walker, L., and D. B. Lowrie. 1981. Killing of *Mycobacterium microti* by immunological activated macrophages. *Nature (London)* **293**:69-70.
- World Health Organization. 1980. Purification of *M. leprae*. Report of the fifth meeting of the scientific working group on the immunology of leprosy (IMMLEP). World Health Organization Technical Report no. TDR/IMMLEP-SWG (5)/80.3. World Health Organization, Geneva.
- Zurbrick, B. G., D. M. Follett, and C. J. Czuprynski. 1988. Cytokine regulation of the intracellular growth of *Mycobacterium paratuberculosis* in bovine monocytes. *Infect. Immun.* **56**:1692-1697.