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NOTES

Modulation of *Mycobacterium lepraemurium* Growth in Murine Macrophages: Beneficial Effect of Tumor Necrosis Factor Alpha and Granulocyte-Macrophage Colony-Stimulating Factor

MICHEL DENIS†

Bioscience I, ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, SK10 4TG, Cheshire, United Kingdom

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***Mycobacterium lepraemurium* grew progressively in monolayers of Proteose Peptone-elicited macrophages from C57BL/6 mice. Treatment of macrophage monolayers with gamma interferon led to an enhancement of growth of *M. lepraemurium* in macrophages. Treatment with tumor necrosis factor alpha or granulocyte-macrophage colony-stimulating factor led to restriction of mycobacterial growth in macrophages.**

Mycobacterium lepraemurium is an obligate intracellular parasite which causes murine leprosy (1). *M. lepraemurium* infects and replicates within cells of the mononuclear phagocyte series. Infection of mice with *M. lepraemurium* is done frequently to provide a model for human leprosy and other chronic mycobacterial infections. There is evidence for genetic control of resistance and susceptibility to infections with *M. lepraemurium* in inbred mice, with the *Ity* (*Lsh*, *Bcg*) gene controlling resistance to infections via the intravenous route (3) and many genetic variables controlling resistance and susceptibility to subcutaneous inoculation (4). It now appears that the *Ity* (*Lsh*, *Bcg*) gene is expressed at the level of the mature macrophage, although the precise mechanism responsible for preventing bacterial growth in Ity^r (Lsh^r, Bcg^r) cells is still unclear (5).

Moreover, the importance of cytokines in delineating resistance to *M. lepraemurium* infections is still to be fully understood. In other mycobacterial infections, gamma interferon (IFN-γ) endows murine macrophages with significant mycobacteriostatic activity (9). The situation is less clear with human macrophages, for which most investigators find that INF-γ increases growth of mycobacteria (8) or has no beneficial effect (18). Tumor necrosis factor alpha (TNF-α) is a cytokine which may increase antimycobacterial activity of human macrophages against *Mycobacterium avium* and *Mycobacterium tuberculosis* (2, 7). Little is known about the importance of cytokines in *M. lepraemurium* infections. A recent report suggests that IFN-γ may have a detrimental role in macrophage resistance to *M. lepraemurium* (14). In that same report, there was also no evidence that interleukin 4 could increase macrophage resistance to this pathogen, although interleukin 4 could abrogate IFN-γ-induced enhancement of mycobacterial growth. Other recent data suggest that interleukin 2 inoculation may provide protection against *M. lepraemurium* infection in vivo (12).

I set out to investigate the ability of macrophages of susceptible C57BL/6 inbred mice to harbor *M. lepraemurium* and to respond to macrophage-activating lymphokines. I chose to investigate the ability of TNF-α and granulocyte-macrophage colony-stimulating factor (GM-CSF) to modulate intracellular mycobacterial growth; both of these cytokines have been shown previously to induce mycobacteriostatic activity in macrophages (2, 20).

Pathogen-free C57BL/6 mice (Charles River, Inc.) were used as a source of macrophages. Proteose Peptone-elicited peritoneal macrophages were collected and purified as described in detail previously (5). Macrophages were adhered to cover slips in six-well plates at 2×10^6 cells per ml in RPMI 1640 fortified with 10% heat-inactivated fetal calf serum and 2 mM glutamine for 24 h before nonadherent cells were washed. Lymphokines, if required, were added 18 h before infection. Recombinant murine IFN-γ was obtained from Genentech (South San Francisco, Calif.), and recombinant mouse TNF-α and GM-CSF were purchased from Genzyme (Boston, Mass.). All cytokines were diluted in pyrogen-free saline. When present, IFN-γ and TNF-α were added 18 h before infection of cells and also throughout infection, whereas GM-CSF was added for 1 week after infection only, since macrophage growth was to be avoided. Medium was changed every week, with fresh cytokines added weekly if required. *M. lepraemurium* was harvested from the livers of systemically infected BALB/c mice by methods described elsewhere in detail (20). Macrophages (ca. 2×10^6, 97% pure, as seen with Giemsa stains) were infected with 5×10^7 bacilli in Hank's balanced salt solution (Gibco, Grand Island, N.Y.) for 2 h. Extracellular bacilli were then washed with Hank's balanced salt solution, and cells were incubated for determined periods in a 37°C incubator with 5% CO_2. Selected cover slips were fixed and stained with carbol fuchsin (14), and the number of acid-fast bacilli was determined by counting 100 macrophages per cover slip. There was little indication of a difference in the phagocytic rate between IFN-γ-treated and untreated C57BL/6 macrophages (Fig. 1). *M. lepraemurium* grew progressively in untreated C57BL/6 macrophages such that seven or eight bacilli were found per cell at 3 weeks after infection, at which time a plateau phase was attained; similar growth of the mouse leprosy bacillus in macrophages has been reported before (1, 13). It was also apparent that IFN-γ enhanced growth of *M. lepraemurium* in a dose-dependent

† Present address: Unité de Recherche, Centre de Pneumologie de l'Hôpital et de l'Université Laval, 2725, Chemin Sainte-Foy, Sainte-Foy, Quebec, Canada G1V 4G5.

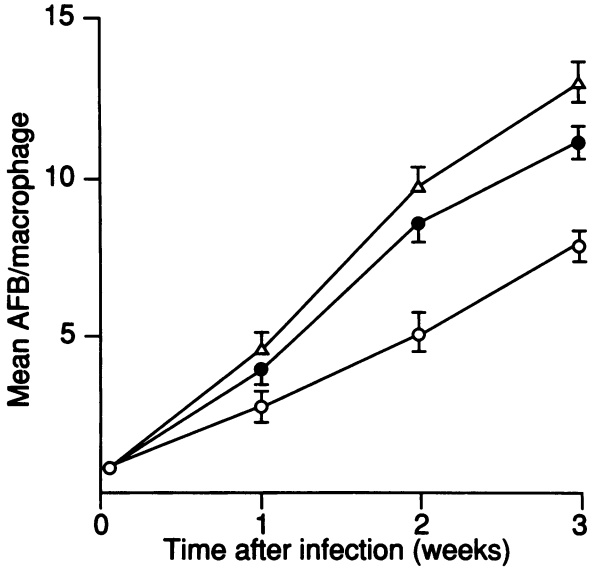

FIG. 1. IFN-γ increases intracellular *M. lepraemurium* growth in murine macrophages. Mouse macrophages were left untreated (○) or were pulsed with 10^2 (●) or 10^3 (△) U of IFN-γ per ml and infected. Lymphokines were present from 18 h before phagocytosis throughout the infection, with fresh IFN-γ added every week. Results ± standard deviations are from triplicate cultures of three experiments (10^3 U versus untreated; $P < 0.05$ at 2 and 3 weeks). AFB, Acid-fast bacilli.

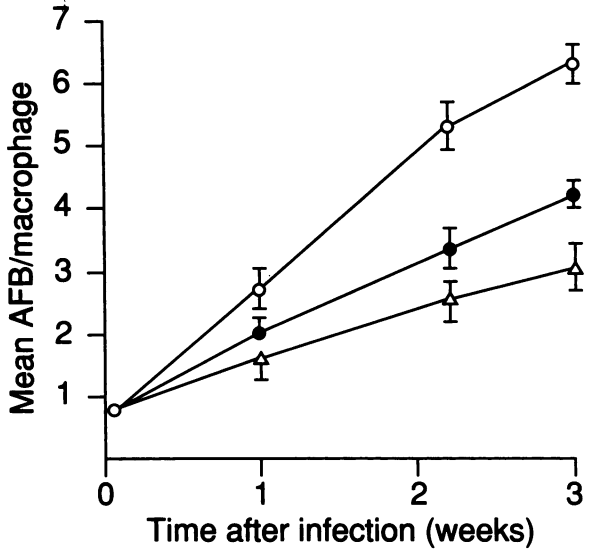

FIG. 2. GM-CSF increases resistance to *M. lepraemurium*. Mouse macrophages were left untreated (○) or were pulsed with 10^2 (●) or 10^3 (△) U of GM-CSF per ml 18 h before infection. Lymphokines were present from 1 h before phagocytosis until up to a week after infection. Results are expressed as in Fig. 1. At 2 and 3 weeks postinfection, all experimental groups were significantly different from each other ($P < 0.01$ by the Student *t* test). AFB, Acid-fast bacilli.

manner such that 13 or 14 bacilli per cell were seen 3 weeks after infection in macrophages treated with IFN-γ at 10^3 U/ml. The difference in growth between IFN-γ-pulsed groups and untreated cells was significant at 2 and 3 weeks ($P < 0.001$, by the Student *t* test).

In the next set of experiments, I set out to investigate the ability of other cytokines to modulate the growth of *M. lepraemurium* in mouse macrophages. Recent data have suggested that GM-CSF may stimulate antimycobacterial activity in bovine monocytes (21) and human monocyte-derived macrophages (5). Accordingly, macrophages were incubated in GM-CSF and allowed to phagocytose *M. lepraemurium*. The growth was then monitored as described previously, with GM-CSF (10^2 or 10^3 U/ml) being present in the first week of infection. When these doses were used, there was no difference in cell number between control monolayers and GM-CSF-treated cells before or after infection, as evaluated by the method of Nakagarawa and Nathan (15) (data not shown). As shown in Fig. 2, 10^2 or 10^3 U of GM-CSF per ml significantly reduced growth of *M. lepraemurium* in C57BL/6 macrophages (reduction of ca. 40 and 60%, respectively, compared with controls at 2 and 3 weeks [the Student *t* test, $P < 0.01$]).

In another set of experiments, I treated monolayers with TNF-α and assessed the ability of cells to modulate *M. lepraemurium* growth intracellularly. Both doses of TNF-α (10^2 or 10^3 U/ml) increased macrophage resistance to *M. lepraemurium* ($P < 0.001$ compared with untreated cells at 2 and 3 weeks after infection). A twofold decrease was apparent at 10^3 U of TNF per ml (Fig. 3). Therefore, my results suggest that GM-CSF and TNF-α have a beneficial effect on growth of murine leprosy bacillus in vitro, whereas IFN-γ may promote the intracellular growth of *M. lepraemurium*, as others have found (14). The ability of IFN-γ to enhance mycobacterial growth in macrophages in certain systems is now well established (8). However, IFN-γ may induce strong bacteriostatic activity against *M. tuberculosis* in mouse bone marrow-derived macrophages (9).

My results, showing that TNF-α may endow macrophages

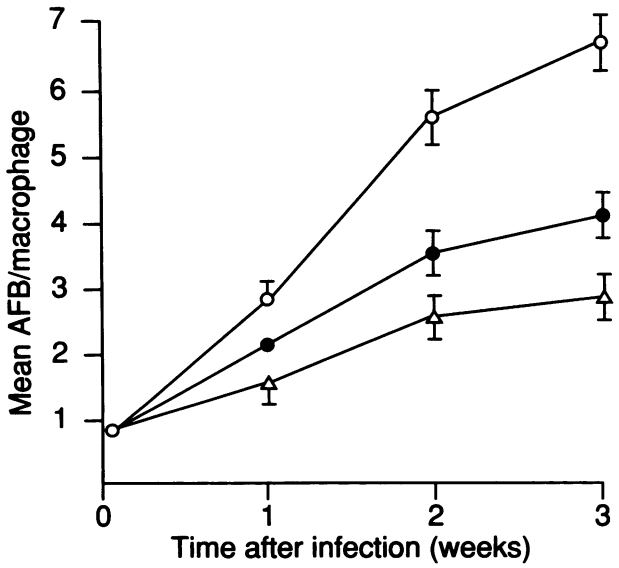

FIG. 3. TNF-α increases resistance to *M. lepraemurium*. Mouse macrophages were left untreated (○) or were pulsed with 10^2 (●) or 10^3 (△) U of TNF-α per ml 18 h before infection. Lymphokines were present from 18 h before phagocytosis throughout the infection. Fresh TNF-α was added every week. Results are expressed as in Fig. 1. At 2 and 3 weeks postinfection, all experimental groups were significantly different from each other ($P < 0.01$ by the Student *t* test).

with the ability to restrict the growth of *M. lepraemurium*, suggest an important role for TNF-α in mediating resistance to mycobacterial infections. Recent results show that TNF-α may endow human macrophages with bacteriostatic ability against *M. avium* (2) and *M. tuberculosis* (7). Moreover, resistance of mice to *Mycobacterium bovis* BCG has been shown to be mediated by TNF-α (13). In that study, it was found that injection of an antibody against TNF-α made the disease significantly worse and prevented the formation of granulomas. Similar experimental protocols have demonstrated the crucial importance of TNF-α in resistance to other intracellular pathogens (11). Furthermore, direct administration of TNF-α may protect against a variety of bacterial insults (10), suggesting the importance of TNF-α in resistance to bacterial pathogens. My own recent results show that resistance to systemic *M. lepraemurium* infection in both resistant and susceptible mice is drastically reduced by depleting endogenous TNF-α (unpublished data). It is also apparent that there is a defect in the production of TNF in patients with lepromatous leprosy, which may partly explain their susceptibility (17). The nature of the antibacterial activity of TNF-α-treated macrophages is still unclear. Although TNF-α may enhance the respiratory burst of macrophages, it is unlikely that reactive oxygen intermediates are involved in macrophage resistance to mycobacteria (9).

The involvement of GM-CSF in resistance to mycobacteria has been shown in two other studies (6, 21). My results with *M. lepraemurium* suggest that this lymphokine induces strong antimycobacterial activity in macrophages. The importance of GM-CSF in endowing resistance to intracellular pathogens has been shown with other parasites, notably *Trypanosoma cruzi* and *Leishmania donovani* (16, 19). As with TNF-α, there is no indication as to the mechanism involved in the increased resistance mediated by GM-CSF.

M. lepraemurium is an obligate intracellular pathogen which is used as a model for the human leprosy bacillus (3, 4). As in the human population, a spectrum of resistance and susceptibility exists in inbred strains of mice. Although many immunological parameters may be involved in the progression of the disease, the ability of macrophages to kill or restrict the growth of the leprosy bacillus must play a crucial role. My results suggest that cytokines play a bidirectional role in macrophage resistance to the mouse leprosy bacillus, with IFN-γ having a negative influence and TNF-α and GM-CSF playing a positive role. It may be that the balance in the production of these cytokines determines the outcome of the disease.

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