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Recombinant Murine Beta Interferon Enhances Resistance of Mice to Systemic *Mycobacterium avium* Infection

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Susceptible BALB/c mice were infected with *Mycobacterium avium* TMC 702. Groups of mice were then infused with 10^4 U (~400 U/h) of murine beta interferon (IFN-β) via a minipump system, and the progression of the infection was assessed. Mice infused with IFN-β showed superior resistance to infection, as determined by reduced bacterial growth in the livers and spleens of infected animals, (1-log reduction in bacterial CFU at 2 months postinfection; $P < 0.001$). This was corroborated by the fact that resident peritoneal macrophages treated with IFN-β in vitro (10^2 U/ml) were more bacteriostatic for *M. avium* TMC 702 than their untreated counterparts. Overall, these findings suggest an important role for IFN-β in mycobacterial infections.

Infections with mycobacteria belonging to the *Mycobacterium avium-M. intracellulare* complex have become an important problem for immunosuppressed patients, including those with AIDS (15). Indeed, *M. avium-M. intracellulare* is the most frequent cause of bacterial infections in AIDS patients in many regions of the world (15). In the tissues of infected susceptible hosts, there is evidence of massive replication of the *M. avium* complex in mononuclear phagocytes. We still have a relatively poor understanding of the mechanisms responsible for host resistance to this class of pathogen (3). Chronic infections with *M. avium* follow a course somewhat similar to leprosy, with little evidence of an effective immune response (19). Suppression in the later stages of the disease is also a characteristic of atypical mycobacterioses, although the exact relevance of this suppression in the progression of the disease is unclear (13). Also, the importance of lymphokines in resistance to the *M. avium* complex is still unclear.

A recent publication from Bermudez and Young (3) reported on the activity of cytokines on macrophage handling of an avirulent *M. avium* strain. These authors suggested that tumor necrosis factor alpha, interleukin-2 (IL-2), IL-4, and granulocyte-macrophage colony-stimulating factor could enhance macrophage killing of an avirulent *M. avium* strain (3). However, the levels of killing reported in these studies are marginal and are not likely to be significant for mycobacteria, microbes that clump readily (6). It appears that interferon gamma (IFN-γ), a classical macrophage-activating molecule (14), may not endow mouse or human macrophages with significant bacteriostatic potential against virulent *M. avium* (22). Similarly, IFN-γ infusion may not enhance the resistance of *M. avium*-infected mice (21). In other mycobacterial infections, it has been shown that IFN-γ may moderately enhance the resistance of mice infected with *M. bovis* BCG (1); it also appears that tumor necrosis factor alpha may be important in *M. bovis* BCG infection (12). IL-2 administration has also been shown to increase host resistance to *Mycobacterium lepraemurium* and *M. bovis* BCG (11).

Although the pivotal role of IFN-γ in enhancing host resistance to infectious agents has been stressed (14), the importance of IFN-α/β is also of note. The pivotal role of IFN-α/β in viral infections has been appreciated (2). IFN-α/β was originally defined and cloned on the basis of its ability to develop antiviral activity in cells (2). However, some interest has been generated in the possibility that IFN-β may have immunomodulating properties in bacterial infections (10). Indeed, IFN-β has been shown to activate monocytes and macrophages to kill *Leishmania major* (16) and to restrict the growth of *Toxoplasma gondii* (17) and *Chlamydia psittaci* (5) in vitro. Moreover, exogenous infusion of IFN-β has been shown to enhance resistance to infection with streptococcal strains (23) and *Listeria monocytogenes* (10). I thus tested the hypothesis that exogenous IFN-β could enhance resistance to *M. avium* infection.

M. avium TMC 702 was used throughout. It was grown in Middlebrook 7H9 broth (Difco Laboratories, Detroit, Mich.) for 21 days. Aliquots were then frozen at −70°C. For infection, bacteria were thawed and sonicated briefly to obtain a dispersed suspension. Bacteria were injected intravenously (i.v.) into specific-pathogen-free BALB/c mice. Infected mice were injected intraperitoneally with osmotic minipumps (Alzet 2002; Alza, Palo Alto, Calif.) filled with 200 μl of recombinant human IFN-β (Triton Biosciences, Alameda, Calif.). Pumps were calibrated to deliver 10^4 U of IFN-β per day (ca. 400 U/h) for the first 30 days after infection. Control mice received pumps containing heat-inactivated cytokines to control for residual endotoxin.

At various times postinfection, mice were killed by exposure to CO_2. The degree of infection was quantified by plating serial dilutions of organ homogenates as described in detail elsewhere (7). The study of the bacteriostatic activity of mouse peritoneal macrophages for *M. avium* TMC 702 was performed as described elsewhere (8). Briefly, the peritoneal cavities of mice were washed with 10 ml of ice-cold RPMI-1640 (GIBCO, Grand Island, N.Y.) and the washes were pooled. Cells were adjusted to 2×10^6 macrophages per ml in Neumann-Tydell medium (GIBCO) fortified with 4 mM glutamine (GIBCO) and 5×10^{-5} M 2-mercaptoethanol (Sigma, St. Louis, Mo.). Cells were plated at 100 μl per well in 96-well plates (Costar, Camnbridge, Mass.). Plates were incubated for 2 h at 37°C in 5% CO_2 to allow adherence. Nonadherent cells were removed by vigorous washings with warm medium. Macrophage monolayers were then pulsed with IFN-β or boiled IFB-β for 18 h. Monolayers were infected with 10^6 *M. avium* for 10 h. Wells were then washed exhaustively with medium to remove extracellular *M. avium*. Plates were then incubated for indicated periods

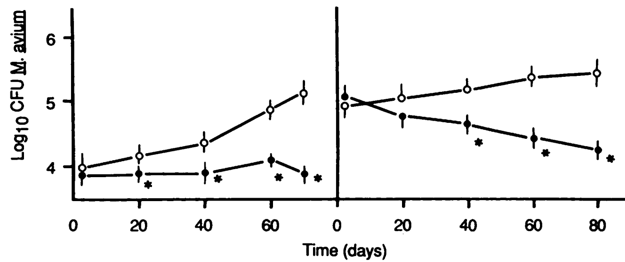

FIG. 1. Growth of *M. avium* TMC 702 in spleens and livers of susceptible BALB/c mice. Mice were injected by the i.v. route with 10^5 CFU of *M. avium*. Bacterial growth was assessed as described in Materials and Methods. Results are shown for growth in the spleens (left panel) and in the livers (right panel) of infected mice. Groups of mice were infused with 10^4 U of IFN-β (●) or heat-inactivated cytokines (○) per day, both delivered by osmotic minipumps. Results are expressed as means ± standard deviations for five mice per datum point. *, $P < 0.05$. Experiments were repeated three times with similar results.

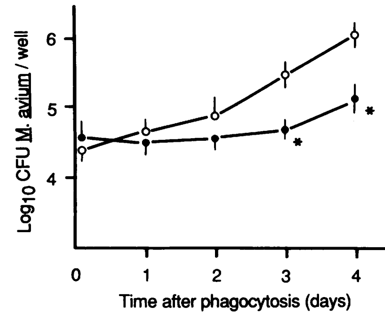

FIG. 2. Growth of *M. avium* TMC 702 in BALB/c murine resident peritoneal macrophages. Cells from BALB/c mice were treated overnight with 10^2 U of IFN-β (●) or treated with boiled IFN-β (○). Results are expressed as means ± standard deviations of triplicate wells. *, $P < 0.05$. Experiments were repeated three times with similar results.

of time at 37°C in 5% CO_2. Cells were lysed with saponin, and lysates were plated on 7H10 agar to evaluate the number of microorganisms (8). The details of the controls used in this assay have been described elsewhere (8).

Figure 1 shows that i.v. injection of 10^5 *M. avium* TMC 702 into BALB/c mice led to progressive growth of the bacteria in the spleens for at least 70 days, and there was no evidence of bacterial elimination in the livers of infected mice. IFN-β infusion, via the minipump system, led to reduced mycobacterial growth in both spleens and livers. IFN-β-treated mice did not allow any net growth of *M. avium* in their spleens (1.2-log difference in CFU at day 70), whereas a small but significant elimination of the bacilli in the tissues (1.0-log difference in CFU at day 80) was seen in the livers. The differences at day 80 in both livers and spleens were highly significant ($P < 0.0001$). These results clearly establish that IFN-β has in vivo antimycobacterial properties.

These results do not, however, suggest a mode of action of IFN-β in vivo; they do not discriminate between a direct or an indirect action of IFN-β. Indeed, IFN-β could be acting via stimulation of the release of other mediators, e.g., IL-1 (4), or acting directly on macrophages infected with *M. avium*.

I tested the latter possibility by monitoring the interaction between macrophages and *M. avium* in vitro and its modulation by IFN-β. As shown in Fig. 2, *M. avium* TMC 702 grew progressively in control macrophage monolayers, with a 1.5-log CFU increase in the number of organisms during the 4 days of in vitro infection. It was also apparent that addition of IFN-β at 10^2 U reduced the growth of *M. avium*, so that at day 4, a 0.8-log difference ($P < 0.01$) in CFU was seen between IFN-β-treated macrophages and control cells. Addition of IFN-β to *M. avium* in the absence of macrophages had no effect on microbes (data not shown). These results clearly show that IFN-β activates the antimycobacterial properties of murine macrophages.

Chronic infections with virulent *M. avium* are an important complication in AIDS and are difficult to manage clinically (15). A better understanding of this type of microbial infection is urgently required. I report here that type 1 interferon, IFN-β, has a beneficial effect on the progression of infection with a virulent *M. avium* strain in a murine model. This heightened resistance was expressed as decreased microbial growth in the spleens and livers of infected mice. Although I have documented that IFN-β may enhance resistance against *M. avium* in mouse macrophages, it remains a possibility that IFN-β was acting partly by stimulating the release of other mediators, such as IL-1 (4). These possibilities deserve further investigation. The mechanism of IFN-β-induced macrophage resistance to *M. avium* was not elucidated. It now appears that activation of mouse macrophages by lymphokines is not dependent on the generation of reactive oxygen species (8, 9). Microbiocidal and microbiostatic mechanisms in activated macrophages independent of the release of reactive oxygen species have been described. They include depletion of essential nutrients (17), enhanced phagosome-lysosome fusion (13), and release of reactive nitrogen intermediates, among others. There is no indication from these studies on the property induced in IFN-β-treated macrophages which restricts the growth of *M. avium* in vitro.

As mentioned above, IFN-β has been used in vivo to increase mouse resistance to *L. monocytogenes* (10). This was shown to correlate in vitro with enhanced macrophage activation, as seen by elevated release of hydrogen peroxide upon triggering with phorbol-myristic acetate (10). Thus, these results suggest an important role for IFN-β in host resistance against intracellular pathogens. A main source of IFN-β in vivo appears to be fibroblasts (2). This alone could explain the fact that nude mice are not more susceptible than athymic mice to *M. avium* infections (19). In any case, this suggests that IFN-β has an important immunotherapeutic potential in mycobacterioses.

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