Failure of an Interferon Inducer to Inhibit Multiplication of *Mycobacterium leprae*[1] (34734)

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Polyinosinic:polycytidylic acid, (I:C) a synthetic double-stranded RNA which is highly active in stimulating interferon production *in vivo* and *in vitro* (1), has recently been shown to inhibit multiplication *in vivo* of several intra- and extracellular pathogens other than viruses including pneumococci, cryptococcus (2), listeria (Remington, J. and Merigan, T. C., unpublished observations) and various gram-negative bacteria (Weinstein, M. J., Waitz, J. A., and Came, P. E., personal communication). It also has been shown to be active *in vivo* against a trachoma agent (Oh, J. O., Ostler, H. B., and Schachter, J., personal communication), *P. berghei* (3), and certain tumors not known to be caused by viruses (4). It therefore seemed of interest to see if treatment with I:C would prevent multiplication of *Mycobacterium leprae* inoculated into the foot pads of mice.

Methods. Crystalline polycytidylic acid and polyinosinic acid were obtained from Miles Laboratories, Inc., Elkhart, Indiana. Equimolar quantities of each were dissolved in phosphate-deficient saline (PDS) (5) and allowed to anneal at room temperature for several hours prior to dilution with PDS to a concentration of 1.5 mg/ml and storage at −20°. Extent of annealing was judged to be adequate by measurement of a hypochromic effect at 260 mμ as well as by assay of antiviral activity in human skin cells in tissue culture (6).

Each of 180 male BALB/c mice was inoculated in the right hind foot pad with 5×10^3 *M. leprae* of a strain originally isolated by C. C. Shepard, National Communicable Disease Center, Atlanta, Georgia, from a patient with lepromatous leprosy and subsequently maintained in mouse passage. 150 μg of I:C in 0.1 ml of PDS were administered by intraperitoneal injection to each of one group of 35 mice three times weekly for 150 days, beginning on the day of inoculation. Each of a group of 25 mice received an intraperitoneal injection of 0.1 ml of PDS without I:C twice weekly. 30 mice were treated with dapsone (4, 4′-diaminodiphenylsulfone, DDS) 0.0001% in the mouse diet for a period of 90 days, beginning 60 days after inoculation. Finally, three groups of 30 mice each, also inoculated with *M. leprae,* served as untreated controls. Harvests of pooled foot pad tissue from four mice were performed at intervals after inoculation; the number of acid-fast bacilli (AFB) per foot pad was determined for each harvest (7, 8).

At intervals during the experiment, two mice from the group to which I:C was administered were exsanguinated, the blood was pooled and the serum was separated. The interferon present in the serum samples was measured in a plaque-reduction assay carried out in L-cell monolayers employing vesicular stomatitis virus (9).

Results. The results of the mouse foot pad harvests are presented in Fig. 1. The bold solid line represents the logarithmic phase of the growth curve of *M. leprae* in the untreated mice. Because one cannot count accurately numbers of organisms smaller than about 4×10^4/foot pad, the lag phase cannot be demonstrated, and one may only infer its existence. The stationary phase of the growth curve has not been shown; this ordi-

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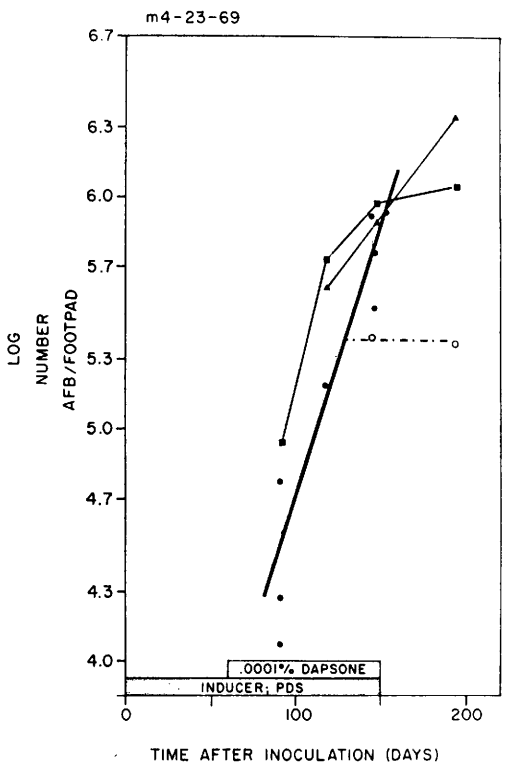

FIG. 1. Multiplication of *M. leprae* in the foot pads of mice, either untreated or treated with I:C, PDS, or dapsone: (●), no treatment; (■), PDS (0.1 ml, ip) twice weekly; (▲), I:C (150 μg in 0.1 ml of PDS, ip) three times weekly; and (○), 0.0001% dapsone in diet.

narily begins soon after bacterial numbers have passed 10^6. This growth curve is similar to many others encountered with this strain of *M. leprae*.

This strain of *M. leprae* is known to be susceptible to dapsone. In this experiment, bacterial multiplication ceased after about 70 days of administration of 0.0001% dapsone in the diet (broken line). Multiplication had not resumed 44 days after dapsone was discontinued. This result is similar to those of previous experiments, in which the effects of dapsone on this strain of *M. leprae* have been studied.

The light solid lines connect the points representing the results of harvests from the inducer-treated and PDS-treated animals. Multiplication of *M. leprae* appears to have proceeded at the same rate in both groups of animals; this rate is probably no different from that in the untreated control mice. Certainly, there is no evidence that treatment with I:C has inhibited bacterial multiplication.

Assay of the samples of serum obtained from the inducer-treated mice demonstrated titers of interferon ranging between 10 and 65 units/4 ml; the highest titer was found in a sample taken 2.5 hr after an injection of I:C.

Discussion. In the experiment reported here, induction of interferon in mice by the administration of the synthetic RNA polyinosinic:polycytidylic acid was not accompanied by inhibition of multiplication of *M. leprae* which had been inoculated into the mouse foot pads. This finding stands in contrast to the demonstration of inhibition of the multiplication *in vivo* of other intracellular pathogens (2–4) with comparable dosages of this polymer. The significance of this finding must await an understanding of the mechanism by which this polyanion acts against agents more complicated than virus. Although interferon itself appears to act against TRIC agents (9, 10) and protozoa (11, 12), it appears not to act on bacteria. The antibacterial effects with I:C observed *in vivo* might be explained by any of a number of possible actions, including: increased antibody response (13); enhanced activity of the reticuloendothelial system (2); increased cell-mediated immunity (14); and activation of macrophages. A difference in the sensitivity of *M. leprae* to one or more of these host resistance mechanisms may underly our failure to observe an inhibitory effect.

Summary. Treatment of mice with the interferon inducer polyinosinic:polycytidylic acid failed to inhibit multiplication of *Mycobacterium leprae* in the mouse foot pad. This same treatment has recently been shown to inhibit multiplication *in vivo* of several intra- and extracellular pathogenic microorganisms.

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