Impairment of Interferon Production in Mice Infected with *Mycobacterium lepraemurium*[1] (36171)

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(Introduced by S. Marcus)

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Human lepromatous leprosy is a chronic intracellular infection that extensively involves the reticuloendothelial system and is associated with a generalized depression of cell-mediated immunity (1, 2). The mechanism of immunodepression in human leprosy is unknown. Experimental infection of rodents by *Mycobacterium lepraemurium* (3), however, is also associated with moderate impairment of the delayed allergic response and, thus, provides a model for investigation of this phenomenon. Leprous rodents are depressed in their ability to develop adjuvant arthritis (4) and contact sensitivity and skin homografts are retained significantly longer than in control animals (5). Anergy in murine leprosy is secondary to the infection itself and appears to be related to the multiplication of *M. lepraemurium* in reticuloendothelial tissue of the spleen, bone marrow, and lymph nodes.

Recent investigations have provided support for the concept that interferon production may represent one feature of the altered reactivity of "immune" lymphocytes or macrophages on re-exposure to an immunizing agent (6–9). Since mononuclear cells within the reticuloendothelial system are considered to be a major source of interferon production, these studies were carried out to determine if parasitization of these cells with mycobacteria might be associated with deficiencies of interferon production in addition to abnormalities of cellular immunity.

Methods. Mice. CF_1 female mice were obtained from Carworth Farms, Inc. All animals were housed under conditions of controlled humidity and temperature and a standardized 12-hr lighting cycle with food and water *ad libitum*.

M. lepraemurium *injection. M. lepraemurium* (Hawaiian strain) was kindly supplied by Dr. Byron Tepper, Leonard Wood Memorial Laboratories, Johns Hopkins University, Baltimore, MD. Six- to 8-week-old animals, weighing approximately 30 g were injected intraperitoneally with 0.6 ml of an inoculum containing 1×10^9 *M. lepraemurium* harvested from pelvic fat pads of previously infected mice. Infected fat pads were removed by sterile technique and 0.1 g of material was homogenized with 3 ml of phosphate-buffered saline (pH 7.2) in a Ten Broeck tissue grinder. Adjustment to an inoculum size of 1×10^9 bacilli was performed after quantitation of bacilli by a modification of the technique of Shepard and McRae (10). Ten microliters of formol milk was placed within a 1 cm^2 circle on a specially prepared microscope slide (Bellco Glass, Inc.) and mixed with an equal volume of homogenate. The preparation was then air dried, fixed by exposure to formaldehyde vapor, and coated with gelatin–phenol (0.5 g of gelatin dissolved in warm 0.5% phenol solution). After acid-fast staining with carbolfuchsin, acid-fast bacilli were counted; and the number per 1 ml of homogenate was calculated by

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the following formula:

$$\frac{\text{AFB counted}}{\text{Fields examined}} \times \frac{1\ \text{cm}^2}{\text{area of microscopic field (cm}^2\text{)}} \times \frac{\text{vol of homogenate}}{0.01\ \text{ml}}.$$

Ten to 12 weeks following infection, the capacity of infected mice to produce interferon in response to a representative series of inducing agents was compared with that of matched normal control animals. The mean count of acid-fast bacilli in the spleens of three mice selected randomly from the infected group was 6.4×10^6/100 mg of tissue. Cultures of spleen suspensions on blood agar, MacConkey agar, Lowenstein-Jensen medium, and in trypticase soy broth and thioglycolate medium did not yield growth of any other organisms.

Induction of interferon. Groups of control and *M. lepraemurium*-infected animals were inoculated with endotoxin (*E. coli* 0111 B4 obtained from Difco Laboratories, Inc.); or synthetic polynucleotide, polyinosinic:polycytidylic acid, poly I:poly C from P-L Laboratories); or Chikungunya virus (CV). Five animals were bled at each sampling time; the sera were harvested, pooled, and assayed as previously described (11) by the 50% plaque inhibition technique employing vesicular stomatitis virus (VSV) as the challenge virus in L-929 cells obtained from the American Type Culture Collection. Each assay was standardized by incorporation of an internal laboratory mouse serum interferon standard which was in turn standardized with the International Standard Mouse Interferon Preparation obtained from the National Institutes of Health. One unit of interferon by our assay system equaled approximately 1.4 units of the International Mouse Standard.

Virus. VSV, Indiana strain, was obtained from the American Type Culture Collection. Stock virus pools were grown in L-929 cells and titered approximately 2×10^6 PFU/ml. Chikungunya virus (CV) was obtained from Dr. Philip Russell, Walter Reed Army Medical Center. Stock virus was prepared from the brains of infected suckling mice, made into a 10% suspension, and assayed by the plaque method in primary chick embryo fibroblasts. Virus pools used for induction of interferon production titered approximately 2×10^7 PFU/ml.

Results. To determine the effect of *M. lepraemurium* on the capacity of the host to produce interferon, infected and matched control animals were exposed to a representative group of inducing agents including CV, poly I:poly C, and *E. coli*, 0111 B4 endotoxin.

Chikungunya virus. Groups of *M. lepraemurium*-infected and control mice were inoculated with approximately 4×10^6 PFU of CV by the intraperitoneal route; and the levels of interferon in the serum were determined 2–3, 6–7, and 20–24 hr later. The mean serum interferon levels from five experiments are summarized in Fig. 1. An 85% reduction was observed at the time of the peak interferon response in these experiments with a mean of approximately 3000 units/ml in control animals compared with 445 units/ml in the serum of *M. lepraemurium*-infected mice. To eliminate the possibility that infection with *M. lepraemurium* might shift the time of the response, samples were also obtained at 2–3 and 20–24 hr fol-

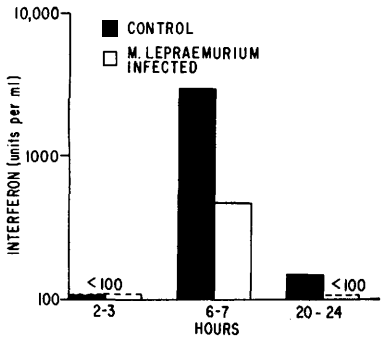

FIG. 1. Mean serum interferon levels in control and *M. lepraemurium*-infected mice after intraperitoneal inoculation with 4×10^6 PFU of Chikungunya virus.

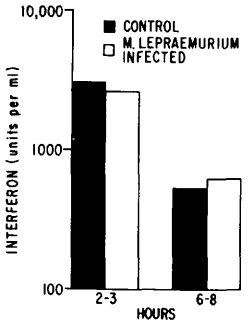

FIG. 2. Mean serum interferon levels in control and *M. lepraemurium*-infected mice after intraperitoneal inoculation with 100 μg of poly I:C.

lowing injection of the inducing agent. At both times, low levels of interferon were observed and no alteration in the pattern of interferon production could be detected. These data suggest that infection with *M. lepraemurium* may adversely affect the interferon response of the host in addition to its suppression of cellular immunity.

Poly I:poly C. In a second series of experiments, 100 μg of poly I:poly C, inoculated by the intraperitoneal route, was used as the inducing agent. In contrast with the reduced capacity of *M. lepraemurium*-infected animals to produce interferon response to CV, normal levels of interferon were found in the serum following induction with synthetic polynucleotides. The mean serum interferon level from four experiments determined 2–3 hr after inoculation with the inducer was 2700 units/ml in the control groups and 2400 units/ml in experimental animals. Six to 8 hr following induction, serum interferon levels were lower in both groups, but again no difference in the mean levels was observed. The pooled data from four experiments are illustrated in Fig. 2. These results indicate that the suppression of interferon synthesis observed following induction with CV is not a generalized phenomenon, but rather reflects specific inducer–host cell interaction.

Endotoxin. The effect of *M. lepraemurium* on the capacity to release "preformed" interferon was evaluated by inoculating groups of infected and control mice intraperitoneally with 100 μg of endotoxin. Samples of serum were obtained for assay of interferon from control and experimental groups at 1.5–2 and 4–5 hr after inoculation. In four experiments, endotoxin consistently stimulated the release of 2- to 4-fold greater levels of interferon in the serum of *M. lepraemurium*-infected animals. The mean serum levels 1.5–2 hr after inoculation in this series of experiments was 175 units/ml in infected animals compared with 80 units/ml in controls. These data are illustrated in Fig. 3. The altered capacity of reticuloendothelial system cells to respond to interferon-inducing agents during the course of experimental infection with *M. lepraemurium* is further documented by these results.

Suppression of interferon in vitro. To more specifically define the effect of *M. lepraemurium* on the reticuloendothelial system, peritoneal exudate cells were harvested from experimental and control animals following stimulation with peptone broth and their capacity to synthesize interferon following exposure to CV *in vitro* was determined. Cells were harvested by washing the peritoneal cavity of anesthetized mice with 5 ml of Eagle's minimum essential media. Differential counts made on Wright-stained smears of exudate indicated a predominance of mononuclear cells with 40–70% lymphocytes and 30–60% macrophages in the cell preparations. The harvested cells were washed, counted, and suspended in Eagle's minimum essential medium with 10% fetal calf serum in 60 mm petri dishes containing 4 ml with 2

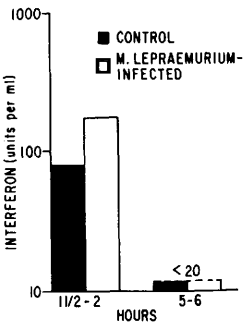

FIG. 3. Mean serum interferon levels in control and *M. lepraemurium*-infected mice after intraperitoneal inoculation with 100 μg of endotoxin (*E. coli*, 0111 B4).

TABLE I. Interferon Production *in vitro* by Peritoneal Exudate Cells from Normal and *M. lepraemurium*-Infected animals Exposed to CV.

Expt.	Interferon (units/ml)	
	Normal	*M. lepraemurium*
I	3000	1000
II	625	80

$\times 10^6$ cells/ml. Groups of four plates of cells from control and infected animals were exposed to approximately 2×10^6 PFU of CV. After 20–24 hr, the media from control and experimental groups were collected, pooled, centrifuged to remove unattached cells, acid-treated, and assayed for interferon activity. The results of two experiments are summarized in Table I. These data indicate that mixed peritoneal cells from mice infected with *M. lepraemurium* do, in fact, reflect the *in vivo* situation and demonstrate a decreased capacity to respond to CV with the production of interferon.

Discussion. This study indicates that chronic infection of mice with *M. lepraemurium* is associated with suppression of interferon production in response to challenge by CV, although the interferon response to synthetic polynucleotides is normal. The complexity of this alteration of host response is further illustrated by the enhanced release of interferon following exposure to endotoxin. Since leprous mice are also impaired in their capacity to mount a delayed allergic response, it would appear that cellular defenses in certain viral infections may be inhibited at multiple levels as a consequence of intracellular infection by *M. lepraemurium*.

The mechanism for suppression of interferon production in murine leprosy is unknown but probably does not require parasitization of individual cells *per se* since examination of peritoneal exudates from infected mice revealed that only occasional monocytic cells contained identifiable bacilli at the time impairment of the interferon response to CV was observed. Recent evidence suggests that interferon production after infection with Chikungunya virus is minimally reduced in mice that have been exposed to 650 R whole-body radiation (12). Thus, it is possible that *M. lepraemurium* may selectively affect the population of relatively radioresistant cells that produce interferon in response to CV. Other possible explanations for the interferon-suppressive effect of murine leprosy might include circulating factors with depressor or toxic activity, impaired or altered patterns of clearance of virus particles by the reticuloendothelial system, and preemption of metabolic pathways by *M. lepraemurium*.

Previous studies have suggested that interferon (13) production was enhanced by infections of the reticuloendothelial system. On the other hand, more recent evidence from our own laboratory has indicated that parasitization by other microorganisms may adversely affect the interferon response of the host (14). Thus, chronic infections involving reticuloendothelial cells may have significant implications for the capacity of the host to respond to subsequent viral infections. It would be of importance, therefore, to determine the interferon-producing capacity of humans with lepromatous leprosy and other chronic infections. Of interest in this regard are a number of uncontrolled clinical reports suggesting that patients with lepromatous leprosy may be more susceptible to variola than normal individuals (15) and may suffer an unusually high incidence of vaccinia gangrenosa or slow resolution of inoculation lesions following vaccination (16).

1. Bullock, W. E., N. Engl. J. Med. **278**, 298 (1968).

2. Bullock, W. E., and Fasal, P., J. Immunol. **106**, 888 (1971).

3. Lowe, J., Int. J. Lepr. **5**, 311, 463 (1937).

4. Bullock, W. E., and Evans, P. D., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **28**, 564 (1969) (abstr.).

5. Ptak, W., Gaugas, J. M., Rees, R. J. W., and Allison, A. C., Clin. Exp. Immunol. **6**, 117 (1970).

6. Glasgow, L. A., J. Bacteriol. **91**, 1285 (1966).

7. Green, J. A., Cooperband, S. R., and Kibrick, S., Science **164**, 1415 (1969).

8. Stinebring, W. R., and Absher, P. M., Ann. N.Y. Acad. Sci. **173**, 714 (1970).

9. Glasgow, L. A., Arch. Intern. Med. **126**, 125 (1970).

10. Shepard, C. C., and McRae, C. H., Int. J. Lepr. **36**, 78 (1968).

11. Murphy, B. R., and Glasgow, L. A., J. Exp.

Med. **127**, 1035 (1968).

12. Glasgow, L. A., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **30** (6), 1846 (1971).

13. Remington, J. S., and Merigan, T. C., Proc. Soc. Exp. Biol. Med. **131**, 1184 (1969).

14. Glasgow, L. A., Odugbemi, T., Dwyer, P., and Ritterson, A. L., Infec. and Immunity **4**(4), 425 (1971).

15. Denney, O. E., and Hopkins, R., Pub. Health Rep. **37**, 3141 (1922).

16. Browne, S. G., and Davis, E. M., Lepr. Rev. **33**, 252 (1962).

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