CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters.

Increased Growth of *Mycobacterium leprae* in Thymectomized-Irradiated Mice after Foot Pad Inoculation

TO THE EDITOR:

Rees and co-workers[1, 2] have recently reported that the growth of *Mycobacterium leprae* is much increased in mice that have been thymectomized and irradiated. When mice were treated in this manner and inoculated in the foot pad, the infections there developed to levels of 10^7 to 10^8 bacteria, i.e., about 10 to 100 times that seen in normal mice.[1] When such treated mice were inoculated intravenously with large numbers of *M. leprae*, a systemic infection developed with special predilection for the feet, ears, and nose and total yields of about 10^{10} bacilli per mouse.[2] Gaugas[3] has recently reported confirmation of the findings with foot pad infections.

We report here our results with this type of experiment. The mice were F1 hybrids of 101 females and C3H males purchased from Cumberland View Farms, Clinton, Tennessee.[4] At the Oak Ridge National Laboratory, 90 mice were thymectomized at about 3 months of age. Nine days later they were x-irradiated with 950 r and injected intravenously with syngeneic bone-marrow cells in a dose of 5×10^6 nucleated cells. In addition 40 nonthymectomized mice were treated with the same dose of x-ray and transfused with 5×10^6 allogeneic marrow cells (from hybrids of C57BL/6 and DBA/2 mice). An additional 40 mice served as untreated controls. All were shipped to the National Communicable Disease Center the day after irradiation and transfusion, and two days later they were all infected with 5×10^3 *M. leprae* (12% of which stained solidly[5]). There were 4 groups of infected mice, treated as follows:

I. Thymectomy and irradiation;
II. Thymectomy and irradiation; after infection tetracycline was given as 0.03 g/l drinking water;
III. Irradiation and foreign marrow transplants;
IV. Normals (infection only).

Survival was good in all groups but III; the number of mice dying during the first 3 months after irradiation was 4, 4, 32, and 1 in the four respective groups.

The results of the counts are given in Figure 1 and Table 1. In the foot pads of normal mice the number of *M. leprae* rose to a plateau level of about $10^{6.0}$, where it remained for the rest of the experiment. The proportion of solidly staining (and therefore presumable viable[5]) bacilli fell to insignificant levels soon after the plateau was reached, and there was no suggestion of the second growth cycle seen in CFW and Chatterjee mice[5, 6]. In the thymectomized-irradiated animals the growth of *M.*

[1] REES, R. J. W. Enhanced susceptibility of thymectomized and irradiated mice to infection with *Mycobacterium leprae*. Nature (London) **211** (1966) 657-658.

[2] REES, R. J. W., WATERS, M. F. R., WEDDELL, A. G. M. and PALMER, E. Experimental lepromatous leprosy. Nature (London) **215** (1967) 599-602.

[3] GAUGAS, J. M. Effect of X-irradiation and thymectomy on the development of *Mycobacterium leprae* infection in mice. British J. Exper. Path. **48** (1967) 417-422.

[4] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U. S. Department of Health, Education, and Welfare.

[5] SHEPARD, C. C. and MCRAE, D. H. *Mycobacterium leprae* in mice: Minimal infectious dose, relationship between staining quality and infectivity, and effect of cortisone. J. Bact. **89** (1965) 365-372.

[6] SHEPARD, C. C. and CHANG, Y. T. Effect of DDS on established infections with *Mycobacterium leprae* in mice. Internat. J. Leprosy **35** (1967) 52-57.

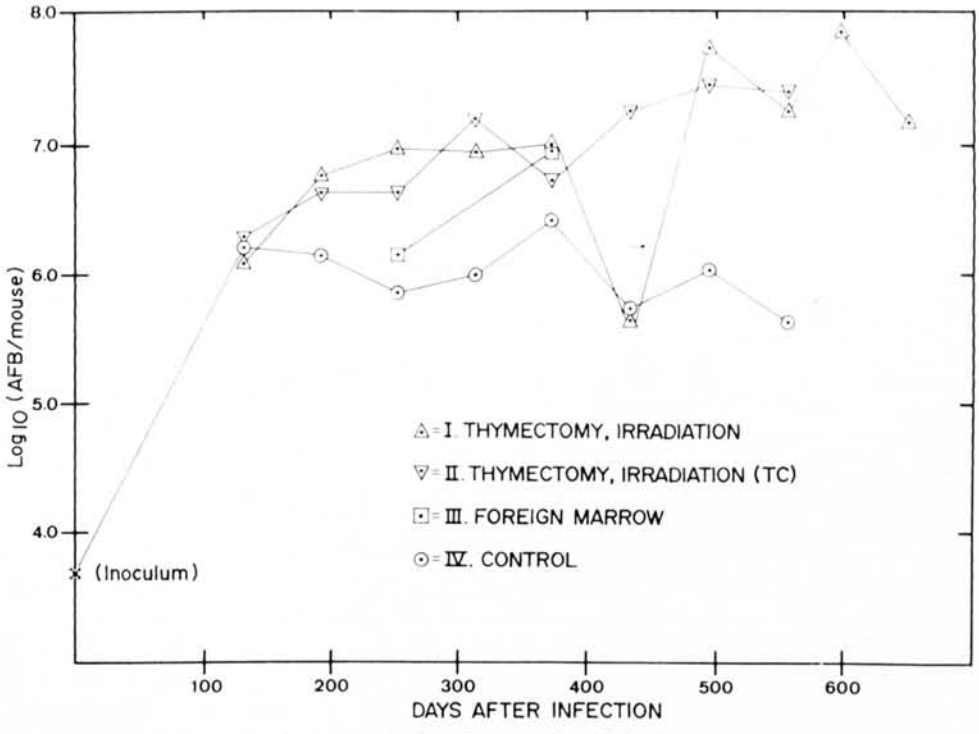

FIG. 1. *M. leprae* infections of the foot pads of thymectomized-irradiated mice. Adult mice were thymectomized, irradiated, and transfused with bone marrow as described in the text. After inoculation with 5 x 10^3 bacilli *M. leprae*, the course of the infection was followed by counts of bacilli in the inoculated foot pads. In Groups I, II, and IV the counts were made on pools of the tissues of 4 mice through day 372, then on pools of 2 mice through day 433, and then on the tissues of 1 mouse; in Group III each harvest represented 2 mice.

TABLE 1. *The proportion of solidly staining bacilli in* M. leprae *infections of the foot pads of thymectomized-irradiated mice.*

Days after infection	Solid ratio (% solidly staining bacilli)			
	I. Thymect. + irrad.	II. Thymect. + irrad. (TC)	III. Foreign marrow	IV. Control
131	28	24		22
193	8	8		<2
252	10	22	14	<2
313	12	9		<2
372	8	2	<2	<2
433	<1	7		<2
494	8	4		<2
557	3	3		<2
599	<1			
651	<1			

leprae during the logarithmic phase of growth[5] was not any more rapid than in the controls, as evidenced by the fact that the first harvests in Groups I, II, and IV were about the same both in total bacillary numbers and in solid ratios. However, during the succeeding period, when growth in the controls had ceased, growth in the thymectomized-irradiated animals continued, although at a gradually slowing rate, until the population approached $10^{8.0}$. The continuing growth was evidenced both by the increasing bacillary numbers and by the continued presence of solid bacilli.

In the mice that had been treated by irradiation and foreign marrow transplants the few survivors took an intermediate position.

Histopathologic studies were carried out. In the right hind (injected) foot the events in normal mice were not different from those seen in CFW and eight other lines of mice,[7] and after 5 months, when the plateau phase had been reached, there was no increase in numbers of bacilli, nor was there evidence of spread. In the thymectomized-irradiated animals the area containing bacilli continued to increase after 5 months, and at 9 months the infection had spread throughout the tissues ventral to the metatarsals.

In the lymph nodes of the thymectomized-irradiated animals there was at first a marked decrease in lymphocytic cells in the medulla. At 6 months, however, there was partial replacement of lymphocytes and after 7 months the nodes were not distinctly different from the controls. Acid-fast bacteria were not present in the regional (flank) lymph nodes of the thymectomized-irradiated mice until 494 days, but they were seen consistently thereafter, sometimes in numbers up to 50 bacteria per microscope field (X562).

Other peripheral tissues were not examined until 433 days, at which time no bacterial spread was detected. At 494 days and thereafter acid-fast bacteria were frequently found in sections from the front feet, the uninoculated hind foot, the ears, and the nose. At 553 days and thereafter acid-fast bacteria were often seen in the bone marrow of inoculated as well as uninoculated feet.

The mouse killed at 651 days had 6.6 x 10^6 acid-fast bacteria in the left ear, 7.4 x 10^6 in the nose, and 1.8 x 10^7 in the right front foot, figures that may be compared to 1.4 x 10^7 in the inoculated (right hind) foot.

One may piece together the observations into the following interpretation, which is based partly on earlier observations[5]. The early bacterial events after inoculation in the thymectomized-irradiated mice were not different from those in normal mice, and multiplication took place in the fibroblasts at a normal rate. When the bacterial population reached about $10^{6.0}$ the normal mice terminated bacterial multiplication by an immune response that resulted in extensive bacterial killing. The treated mice, however, allowed multiplication to proceed at a reducing rate until the population reached a more or less stationary level near $10^{7.0}$. This was about the time that the lymph nodes were repopulated. After 450 days there was further bacterial increase in the inoculated foot and the infection generalized to the other feet, the ears, and nose and the bone marrow.

Nerve invasion has been observed only infrequently in the infected foot pad of normal CFW's,[8] and the other strains studied[7]. In the present study nerve invasion was not noted in the mice killed early. In those killed after 300 days, however, nerve invasion became increasingly prominent both in the injected foot pad and in the areas to which the infection had spread.

The location of the bacilli in the inoculated feet of the thymectomized-irradiated mice was apparently different from that observed by Rees, *et al.*,[1, 2] in that there was no preferential concentration of bacilli in the muscle. Bacilli were found throughout the muscles, but the number per unit

[7] SHEPARD, C. C. and HABAS, J. A. Relation of infection to tissue temperature in mice infected with *Mycobacterium marinum* and *Mycobacterium leprae*. *J. Bact.* **93** (1967) 790-796.

[8] SHEPARD, C. C. *Mycobacterium leprae* in mouse foot pads. Anais VIIIth Congr. Leprol., Rio de Janeiro, September 1963, pp. 333-340. (*Abstract in* Internat. J. Leprosy **31** (1963) 609-610.)

area was no greater there than in subcutaneous tissues and connective tissue spaces between the muscle bundles. In the feet to which the infection had spread, the bacillary location was similar. In the ears the location was also subcutaneous and not in muscle.

In our study the proportion of solidly staining bacilli was lower than that reported by Rees, *et al.*,[1, 2] and the relative difference between treated and control mice was much more pronounced. Possible explanations for the apparent discrepancy include differences in lines of mice, and in the technic of determining solid ratios.

In summary, we have confirmed that when thymectomized-irradiated mice are infected in the foot pads the local growth of *M. leprae* in the foot pads is distinctly increased over that seen in controls. In addition there was bacterial spread to the other feet, the ears, and nose in mice followed for long periods. Our reason for reporting this confirmation is to emphasize that this technic, or other similar ones, appears to offer new approaches to the study of the immunology and pathogenesis of leprosy, and to provide a means for maintaining a laboratory supply of increased numbers of *M. leprae* in high viability.

CHARLES C. SHEPARD[9]
AND
CHARLES C. CONGDON[10]

6 February 1968

[9] National Communicable Disease Center, Bureau of Disease Prevention and Environmental Control, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia 30333.

[10] Biology Division, Oak Ridge National Laboratory (operated by Union Carbide Corporation for the U. S. Atomic Energy Commission), Oak Ridge, Tennessee 37830.