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Effects of *Propionibacterium acnes* Treatment on the Course of *Mycobacterium leprae* Infection in Mice

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Studies were carried out to determine the effects of treatment with killed suspensions of *Propionibacterium acnes* (formerly designated *Corynebacterium parvum*) on the course of *Mycobacterium leprae* infection in mice. Systemic (intravenous or intraperitoneal) treatment with *P. acnes* failed to significantly alter the growth of *M. leprae* in the mouse footpad. In contrast, injections of *P. acnes* directly into the infected footpad markedly inhibited the growth of the leprosy bacilli regardless of whether the local treatments were administered before infection or 3 months after infection with *M. leprae*. The effects of local treatment with *P. acnes* appeared to be bactericidal and not merely bacteriostatic. Clearance of the organism from the tissues was not enhanced by *P. acnes* treatment.

A large body of evidence attests to the immunopotentiating effects of treatment with suspensions of killed *Propionibacterium acnes* (formerly designated *Corynebacterium parvum* [5]). In mice, treatment with *P. acnes* markedly enhances the function of the reticuloendothelial system (2, 11), increases host resistance to tumors (11, 14, 17, 20–22), and enhances their capacity to resist infection with a variety of pathogenic organisms including *Salmonella enteritidis* (3), *Bordetella pertusis* (1), *Brucella abortus* (1), *Plasmodium berghei* (18), *Toxoplasma gondii* (28), and *Listeria monocytogenes* (28).

The present studies were carried out to determine the effects of systemic and local treatment with *P. acnes* on the growth of *Mycobacterium leprae* in the mouse footpad.

MATERIALS AND METHODS

Mice. Locally bred BALB/c mice were used. All of the mice were females and weighed 18 to 22 g at the start of each experiment.

P. acnes. A killed suspension of *P. acnes* (*C. parvum*, lot no. CA749, 7 mg/ml dry weight) was kindly provided by Richard Tuttle (Burroughs-Wellcome Co., Research Triangle Park, N.C.). *P. acnes* treatments were administered intravenously (i.v.), intraperitoneally (i.p.), or subcutaneously (s.c.) in the groin area or s.c. into the hind footpad.

***M. leprae* infection.** Mice were inoculated in the right or both hind footpads (RHF and BHF, respectively [LHF = left hind footpad]) with 5×10^3 *M. leprae*, and the course of infection was followed at intervals by harvest and enumeration of acid-fast bacilli (AFB), using methods described previously (23, 25). When single footpad harvests were carried out, a statistical analysis of the difference between groups was assessed by the two-sample rank test.

Viability of *M. leprae*. To determine the bactericidal effects of local injections of *P. acnes* on *M. leprae* in the treated footpad, the proportion of viable organisms was calculated by the most probable number (MPN) technique. Briefly, at the appropriate time after local footpad treatment (for 8 days in the experiments described below), *M. leprae* were harvested from pools of footpad tissue from control and treated mice, counted, and serial 10-fold dilutions were prepared to contain 5,000, 500, 50, or 5 organisms per 0.03 ml of inoculum. Both hind feet of groups of 10 passage mice were then inoculated with each dilution. One year later, single foot harvests were performed, starting with passage animals which had been inoculated with 50 *M. leprae* organisms. The criterion for multiplication of the inoculum was the presence of at least 5×10^4 AFB/footpad. If multiplication had occurred in all 10 feet, single footpad harvests were performed on passage mice inoculated with the higher dilution of five organisms. If evidence of multiplication was lacking in all or some of the mice from both of these groups, single footpad harvests were carried out in the group of passage mice inoculated with 500 organisms. The proportion of viable *M. leprae* was calculated by the MPN technique developed by Halvorson and Ziegler (12), modified by deMan (8), and adapted for *M. leprae* by Colston et al. (4). Welch et al. (29) have provided a recent detailed description of this technique as employed to measure the viability of *M. leprae*.

RESULTS

Effects of *P. acnes* treatment on growth of *M. leprae*. In the experiment shown in Table 1, two groups of mice were infected in BHF with *M. leprae* on day 0. On day 82 after infection, the

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TABLE 1. Effects of local treatment with *P. acnes* on growth of *M. leprae* in the mouse footpad

Treatment[a]	No. of AFB[b]	
	LHF	RHF
Saline	2.3×10^5	3.9×10^5
P. acnes	2.0×10^5	6.0×10^3

[a] Saline (0.05 ml) or *P. acnes* (350 μg) was injected into the RHF on day 82 of infection.

[b] Harvest was performed at day 152 after infection. Number of AFB/footpad (pool of five footpads).

experimental group was injected in the RHF with 350 μg of *P. acnes*. Harvests performed on day 152 revealed a marked reduction in the number of *M. leprae* in the treated (RHF) but not the untreated contralateral (LHF) footpad. Since these results suggested that local treatment with *P. acnes* killed or inhibited the growth of *M. leprae*, a more comprehensive experiment was performed in which mice infected with *M. leprae* in BHF received single or multiple *P. acnes* treatments by a variety of routes. Thus, in the experiment shown in Fig. 1, at 92 days after infection different groups were treated with a single dose of *P. acnes* administered s.c. in the groin (group B), i.p. (group D), i.v. (group F), or locally in the RHF (group H). Harvest and quantitation of AFB were carried out at day 140 after infection. Compared with controls, s.c. treatment with *P. acnes* was without effect on *M. leprae* growth (Fig. 1, group B). A single i.p. or i.v. treatment with *P. acnes* appeared to afford some protection against the growth of *M. leprae* as shown by fewer AFB in both the LHF and RHF (Fig. 1, groups D and F). However, as these data represent harvests of pooled footpad tissues, the significance of these apparent differences was not tested. As seen in group H, local injection of *P. acnes* into the infected RHF had a marked effect on the growth of *M. leprae* in the treated footpad, with little or no effect on infection in the contralateral LHF. An attempt was also made in this experiment to determine whether a booster treatment of *P. acnes* in the RHF might be more effective in affording resistance to *M. leprae* than would a single treatment. Thus, groups C, E, and G received two treatments, the first administered s.c., i.p., or i.v. at day 92 and the second administered into the RHF on day 99. Regardless of the route of the initial treatment, the local footpad treatments with *P. acnes* had a marked effect (Fig. 1). When harvested at 140 days the number of *M. leprae* in the treated RHF in groups C, E, and G was below the counting threshold for detectable AFB in pooled footpad tissues. In group J the primary and secondary treatments were given in the RHF and LHF on days 1 and 8 after infection, respectively. Harvest at 140 days revealed that in BHF the numbers of AFB were at or below the threshold of counting accuracy for single footpad harvests (Fig. 1, group J).

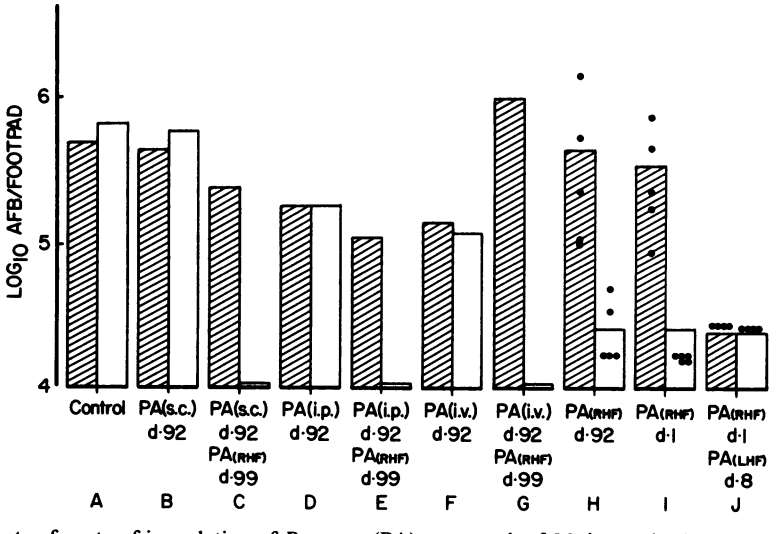

FIG. 1. Effects of route of inoculation of *P. acnes* (PA) on growth of *M. leprae* in the mouse footpad. Mice were infected in BHF with *M. leprae* on day (d) 0, and groups of five mice were each treated with *P. acnes* by various routes (700 μg s.c., i.p., or i.v.; 350 μg in the RHF) on the indicated days. Footpads were harvested on day 140 after infection. Hatched bars, LHF; open bars, RHF. Bars represent the number of *M. leprae* in pooled footpads (groups A through G) or the mean number of *M. leprae* in single footpad harvests ([●] groups H-J).

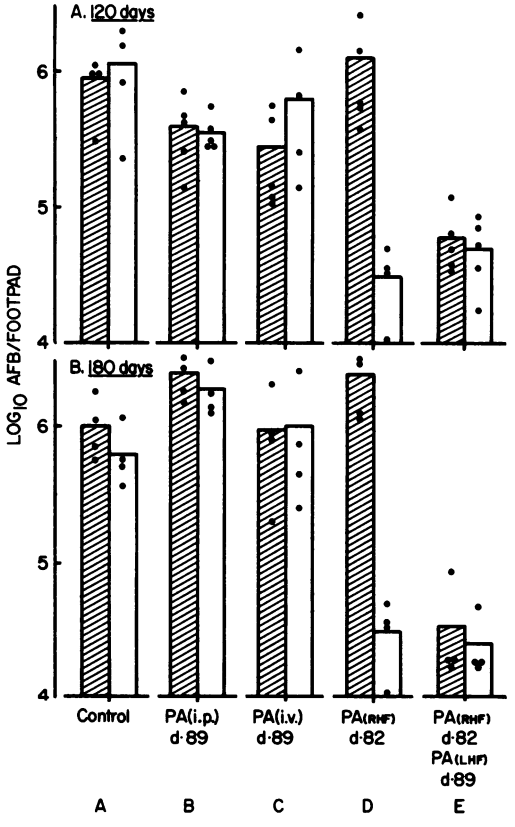

FIG. 2. Effects of route of inoculation of *P. acnes* (PA) on growth of *M. leprae* in the mouse footpad. Mice were infected in BHF with *M. leprae* on day (d) 0, and were treated with *P. acnes* by various routes (700 μg i.p. or i.v.; 350 μg in the RHF) on the indicated days. Footpads were harvested 120 (A) or 180 (B) days after infection. Hatched bars, LHF; open bars, RHF. Bars represent the mean number of *M. leprae* in single footpad harvests (●).

In a subsequent experiment, *P. acnes* was administered i.p., i.v., or locally in the RHF on day 89 after infection. Since the data obtained in the previous experiment (Fig. 1) suggested that i.p. and i.v. administration of *P. acnes* might afford partial protection against the growth of *M. leprae*, the experiment shown in Fig. 2 was designed to confirm the presence of such partial protection and to determine whether such apparent protection would persist (i.e., whether it was a bacteriostatic or a bactericidal effect). Harvests were thus carried out at 120 days as well as 180 days after infection. To accommodate statistical analysis, single footpad harvests were performed. As shown in groups B and C in Fig. 2A, the mean number of AFB per footpad after i.p. or i.v. treatment with *P. acnes* was lower than that of controls, but did not differ significantly ($P > 0.05$). However, after local treatments in the RHF (Fig. 2A, group D), the marked reduction in AFB did differ significantly ($P < 0.05$) from the number of AFB in either the untreated opposite footpad (Group D, LHF) or the footpads of control mice (group A). As seen in group E, treatment of BHF resulted in significantly ($P < 0.05$) fewer AFB in BHF at 120 days after infection. When the second harvest was performed at 180 days (Fig. 2B), there was no evidence of protection in the groups treated i.p. or i.v. (groups B and C, respectively), but the effects of local treatments were even more apparent (groups D and E). These latter results suggested that direct footpad treatment with *P. acnes* induced either a prolonged local inhibition of growth of *M. leprae* or resulted in the local killing of the organism.

Effects of *P. acnes* on clearance of *M. leprae*. Shown in Fig. 3 are the results of two experiments performed to determine whether local treatment with *P. acnes* had an effect on the clearance of *M. leprae* from the infected footpad. Mice infected in BHF 237 days previously were treated in the RHF with either saline or *P. acnes* (Fig. 3A, groups A and B, respectively) or were treated with *P. acnes* in the RHF on day 237 and the LHF on day 244. Twenty-eight days later, single footpad harvests were performed and revealed that local treatment with *P. acnes*

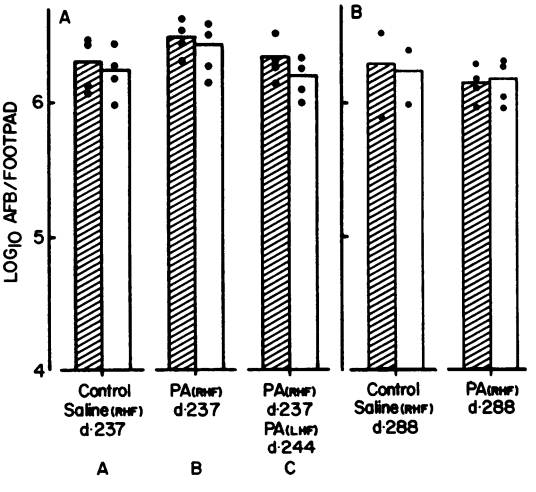

FIG. 3. Effects of local (footpad) treatment with *P. acnes* (PA) on clearance of *M. leprae*. (A) Mice were injected in the RHF or LHF with 350 μg of *P. acnes* on the indicated days. Single footpad harvests were performed on day 272 after infection. (B) *P. acnes* treatment was administered on day 288, and single footpad harvests were performed on day 316 after infection. Bars represent the mean number of *M. leprae* in single footpad harvests (●).

did not appear to have an effect on the clearance of AFB from the footpad (Fig. 3A). The results observed in a similar experiment (Fig. 3B) corroborate these findings. Local administration of *P. acnes* into the footpads of mice infected 316 days previously did not enhance the clearance of *M. leprae* from these tissues.

Effects of *P. acnes* on viability of *M. leprae*. Two experiments were performed to determine the short-term effects of local *P. acnes* treatment on the viability of *M. leprae* in the treated footpad. In the first study 10 mice were used in which footpad growth had plateaued at 1.5×10^6 organisms per footpad (day 152). Half of these animals were treated in the RHF with 350 μg of *P. acnes*; the remaining five mice were treated with saline. To determine the bactericidal effects of local injections of *P. acnes* on *M. leprae* in the treated footpad, the proportion of viable organisms was calculated by the MPN technique. Calculation of the MPN for treated and control groups revealed that within 8 days after administration of a single local treatment with *P. acnes* into the infected footpad, 98.8% of the organisms in that footpad were no longer viable. In a repeat experiment, calculation of the MPN of viable *M. leprae* revealed that >92% were killed within 7 days of local treatment with *P. acnes*.

DISCUSSION

The doubling time of *M. leprae* in the infected mouse footpad is approximately 2 weeks during the logarithmic phase of growth (16). In immunologically competent mice, growth ceases when the number of *M. leprae* plateaus at a million organisms per footpad (19, 23, 27). This prolonged cycle of growth allowed ample opportunity to study the prophylactic and therapeutic effects of systemic and local *P. acnes* treatment on *M. leprae* infection.

Treatment with *P. acnes* has been shown to markedly enhance reticuloendothelial system function (2, 11), host resistance to neoplasia (11, 14, 17, 20–22), and resistance to infection with a wide spectrum of pathogenic organisms including *S. enteritidis* (3), *Bordetella pertusis* (1), *L. monocytogenes* (28), *Brucella abortus* (1), *Plasmodium berghei* (18), and *T. gondii* (28). The present report extends these observations to include *P. acnes*-induced resistance to infection with the leprosy bacillus.

Although local administration of *P. acnes* into the infected footpad resulted in the marked inhibition or killing of *M. leprae*, other routes of administration proved to be ineffective. Attempts to determine whether a local (footpad) booster injection of *P. acnes* would surpass the effects of a single treatment were inconclusive since a single injection of *P. acnes* into the infected footpad provided almost complete protection.

Although growth of *M. leprae* plateaus at a million organisms per footpad, leprosy bacilli persist long after the cessation of growth (24–27). The recent study by Welch et al. (29) presented direct evidence that, after cessation of growth, the proportion of viable *M. leprae* in the footpad decreased at a rate equivalent to the loss of 2.7% of the remaining viable leprosy bacilli per day. Thus, although the number of AFB remains relatively constant for many months, the viability of these organisms diminishes rapidly. In the present study, local injection of *P. acnes* into the infected footpads, several months after growth of the leprosy bacillus had plateaued, failed to reduce the number of AFB. However, although clearance of AFB from the footpad tissues was not enhanced, local injections of *P. acnes* did appear to result in the rapid killing of leprosy bacilli as shown in two experiments by subinoculation and calculation of the MPN of viable organisms. Alternatively, the apparent rapid killing of *M. leprae* in the treated footpads could have resulted from inhibition of growth of *M. leprae* in the recipient animals due to the carry-over of a very slight amount of *P. acnes* that may have persisted in the footpads of the treated mice.

The mechanisms underlying the enhanced resistance to *M. leprae* afforded by *P. acnes* treatment were not addressed directly in the present report, but a wealth of information suggests that the enhanced microbicidal capacity of activated macrophages may have been responsible (3, 9, 28, 30). The use of *P. acnes* for the activation of macrophages in a localized anatomical compartment is highly dependent upon the route of administration. There are no clear examples of the effects of *P. acnes* treatment on local infections, although histopathological studies have revealed the accumulation of large numbers of lymphocytes and macrophages in the tissues at the site of tumor regression mediated by local *P. acnes* injection into the footpad (17).

Others have explored the effects of nonspecific immunotherapy on mouse footpad infections with *M. leprae*. As shown by Shepard and his colleagues (26), injections with living or killed BCG afforded resistance to *M. leprae* growth. This protection may have been attributable to specific antigens shared by these two species of mycobacteria. Scott and Bomford (22) found that larger doses of BCG than those of *P. acnes* were required to produce similar degrees of tumor immunity. Chronic infection of mice with the obligate intracellular protozoa *T. gondii* or *Besnoitia jellisoni* also induced a marked resistance to *M. leprae* growth (15). Resistance was

greatly enhanced by local (footpad) booster injections of specific protozoal antigen. Graft versus host reactions, induced by injection of parental spleen cells into F_1 hybrids, provided only moderate protection against *M. leprae* challenge (26), although allogeneic spleen cells had a protective effect when injected locally into the infected footpad. Shepard et al. (27) also studied the effects of levamisole, an antihelminthic drug with remarkable immunopotentiating properties, and found that treatment failed to enhance host resistance to *M. leprae* in mice. Finally, Delville and Jacques (6, 7) showed that local and systemic injections of glucan (β-1,3-glucosidic polyglucose), a potent stimulator of the reticuloendothelial system, afforded protection to *M. leprae* growth in the mouse footpad.

Of special interest in the present report was the marked effectiveness of local treatment with *P. acnes* regardless of whether it was administered immediately before (1 week) or well after (3 months) *M. leprae* infection had been initiated. In contrast, enhanced resistance to other infectious agents (28) or tumors (21) was optimum only if *P. acnes* treatment was administered before or very shortly after challenge. The prolonged course and localized nature of *M. leprae* infection are probably important factors in the success of *P. acnes* immunotherapy in the mouse model for leprosy.

Experimental cancer immunotherapeutic measures which employ treatment with *P. acnes* are being carried out in humans and elicit only minor signs of toxicity after local routes of administration (10, 13). Whether nonspecific immunotherapeutic measures will ever be employed to treat human leprosy or whether such studies merely are a means of understanding host resistance to the leprosy bacillus remains to be determined.

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