

Lack of Protection Afforded by Ribonucleic Acid Preparations from *Mycobacterium tuberculosis* Against *Mycobacterium leprae* Infections in Mice

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Mycobacterial ribonucleic acid preparations from H37Ra, an attenuated strain of *Mycobacterium tuberculosis*, provide their usual marked protection against *M. tuberculosis* challenge; however, they provided no protection against *Mycobacterium leprae* challenge. Suspensions of intact H37Ra were not effective against *M. leprae*. Suspensions of BCG gave their usual distinct protection against *M. leprae* challenge.

Mycobacterial ribonucleic acid (myc RNA) preparations from the attenuated H37Ra strain of *Mycobacterium tuberculosis* immunize mice against challenge with virulent strains of *M. tuberculosis* (13). The preparations are as effective as viable H37Ra cells, on the basis of the amount of RNA present (13). Homologous ribosomal or RNA preparations have proved to be immunogenical against *Salmonella typhimurium* (10), *Staphylococcus aureus* (11), *Pseudomonas aeruginosa* (11), *Diplococcus pneumoniae* (9), *Neisseria meningitidis* (8), and *Histoplasma capsulatum* (R. P. Tewari, D. Sharma, and R. LaFemina, Fed. Proc. 35: 228, 1976).

Immunization against *Mycobacterium leprae* infections in the footpads of mice can be accomplished by the injection of viable suspensions of BCG strains of *Mycobacterium bovis* and several other mycobacteria (3, 5). It was of interest, therefore, to study the effect of myc RNA on the *M. leprae* infections.

MATERIALS AND METHODS

The myc RNA and suspension of H37Ra were prepared in Chicago, and the tests to determine the potency of the myc RNA against *M. tuberculosis* were carried out there. The tests to determine the potency of the myc RNA against *M. leprae* were carried out in Atlanta. The vaccine materials were shipped under refrigeration by air. To detect possible deterioration during shipment, a portion of the myc RNA shipped to the Atlanta laboratory was immediately returned to Chicago. The myc RNA was 1 day old when inoculated into mice for its first test against *M. tuberculosis* and 3 days old when inoculated for its second test; it was 2 days old when inoculated into mice for its test against *M. leprae*.

The preparation of myc RNA has been described (13). In brief, H37Ra cultures were grown on modi-

fied Proskauer and Beck medium, and 2-week-old cells were mechanically ruptured. The RNA was purified ultracentrifugally, standardized by its optical density at 260 nm, and incorporated into Freund incomplete adjuvant before shipment. For the tests against *M. tuberculosis* challenge, the mice were vaccinated intraperitoneally with 0.4 ml and challenged intravenously 28 days later with 1.0 mg of the H37Rv strain. Those that survived 30 days were considered immune.

The tests against *M. leprae* were carried out as described (3, 7). In short, 28 days after vaccination the mice were challenged in the footpad with 5,000 *M. leprae* bacilli of a mouse passage strain. In unvaccinated controls, the number of *M. leprae* reached microscopically countable levels about 90 days after infection and rose logarithmically until it reached the plateau level, just over $10^{6.0}$ *M. leprae* per mouse, at about 160 days. Harvests of footpad tissues for microscopic counts of *M. leprae* were then carried out on eight individual mice in all vaccinated and control groups; the harvests were repeated 90 days later. The statistical significance of the differences between groups was assessed by the two-sample rank test, a nonparametric test; the distribution of counts within groups is usually not normal. As a measure of the effect of intradermal vaccines, the regional (right inguinal) lymph nodes of the mice given intradermal injections and one control group were measured at the time of harvest. The measurements were made through the intact skin (closely clipped and wetted with alcohol) so that they would be comparable to measurements in living mice (5, 7). Two measurements at right angles to each other were recorded; the average of the two is presented in the figure. The lymphatic drainage from the footpad does not involve the inguinal lymph nodes.

The myc RNA was tested in three different amounts, 50, 5, and 0.5 μg , respectively (Fig. 1). The injections were made intraperitoneally in volumes of 0.4 ml. All of these had been incorporated into Freund incomplete adjuvant, and a control group received the adjuvant plus water only. One group

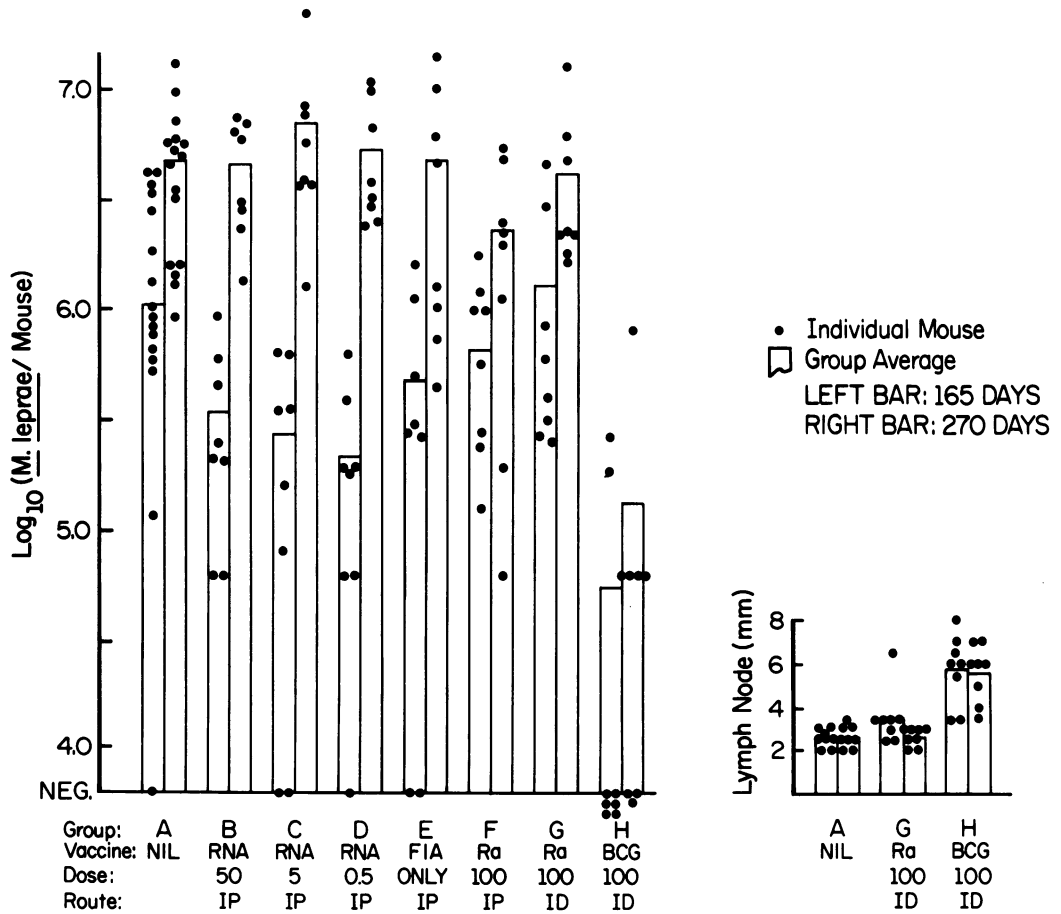


FIG. 1. Results of challenge of vaccinated mice with *M. leprae*. The numbers of *M. leprae* harvested from individual mice near the end of the logarithmic phase in the controls (165 days) and again 90 days later are given on the left. In certain groups, the lymph nodes regional to the intradermal (flank) vaccination were measured at harvest, and these measurements are shown on the right. Abbreviations: RNA, myc RNA; FIA, Freund incomplete adjuvant; Ra, H37Ra; IP, intraperitoneal; and ID, intradermal. The dose of vaccine is given in micrograms.

received H37Ra intraperitoneally in saline suspension in an amount estimated to provide approximately 5 μ g of RNA (approximately 10^6 viable cells); this was given in a volume of 0.2 ml. Another group received the same amount of H37Ra intradermally in 0.01 ml. Another group received a standard suspension of BCG, known from previous experiments to be effective in the amount used, 100 μ g (wet weight; 10^7 bacilli, approximately 10^6 colony-forming units; reference 5) in 0.01 ml intradermally. Two control groups (no vaccine) were included, one at the beginning of the experiment and one at the end. The harvests in the two control groups did not differ significantly, and the control values are combined for the figure and for the statistical evaluations.

To monitor the growth of *M. leprae* in the controls after challenge, four mice were killed at intervals for bacillary counts in the pooled harvest. The \log_{10} 's

of the resulting counts were <4.2 at 63 days, 4.8 at 92 days, 5.53 at 123 days, and 6.26 at 161 days. Harvests were then carried out in individual mice in all the groups, with the results shown in Fig. 1. The plateau level in this experiment was somewhat higher than usual; therefore, the second harvests are higher than the first.

RESULTS

The myc RNA provided no significant protection against *M. leprae* (Fig. 1). In the second harvests, the adjuvant only (group E) provided borderline protection ($P = 0.04$). H37Ra afforded no protection, whether given intraperitoneally or intradermally. The BCG showed its usual distinct protection ($P < 0.001$).

The inguinal lymph nodes (regional to the vaccination site) were measured at the time of harvest in appropriate groups; the results are given on the right in Fig. 1. H37Ra did not cause significant enlargements of the lymph node; however, the animal with a 6.5-mm node and a harvest of $10^{5.5}$ *M. leprae*, i.e., the third lowest value, was the one exception. BCG produced its usual distinct and consistent regional lymph node enlargement.

The results of the *M. tuberculosis* challenge are given in Table 1. The myc RNA gave distinct protection. The protection provided was somewhat lower than usual for 5 and 0.5 μg of myc RNA before shipment, perhaps because of the poor condition of some of the mice. With the possible exception of these two groups, the protection provided was within the expected limits of variation between batches of myc RNA. There was no indication that the myc RNA deteriorated during shipment.

DISCUSSION

We do not know why the myc RNA did not provide protection against *M. leprae*, even though it did provide its usual marked protection against *M. tuberculosis*. Many explanations seem possible; here we mention some of the more obvious ones.

M. leprae and *M. tuberculosis*, of course, differ in antigenic specificity. All mycobacteria share some antigens, but *M. leprae* is not

closely related antigenically to *M. tuberculosis* (2).

There are a number of probably important differences in the two tests for vaccine potency. With the *M. leprae* challenge, the protection is probably exerted chiefly during the onset of the logarithmic phase, i.e., 120 to 180 days after vaccination; even when given after the challenge, BCG vaccine has full activity against *M. leprae* infection (4, 7). In contrast, the entire test with the *M. tuberculosis* challenge is completed within 2 months. Another difference is that the *M. leprae* challenge contains only 5,000 bacilli, and the growth of the bacilli after challenge is much slower (doubling time, 12.5 days in controls); the *M. tuberculosis* challenge contains 1 mg of H37Rv (about 10^7 bacilli), and the growth of the bacilli in the controls has a doubling time of about 1 day. Still another difference of probable importance is that the route of administration of the vaccine and the challenge for *M. leprae* is different from that for *M. tuberculosis*; as a result, the immunizing and challenge materials in the two vaccine tests attain quite different anatomical distributions.

We did not expect the H37Ra suspension to be ineffective against *M. leprae*. Earlier work, carried out less quantitatively, has provided evidence that R1Rv, a strain of *M. tuberculosis* that is less attenuated than H37Ra, afforded protection against *M. leprae* in mice (3). Moreover, heat-killed virulent *M. tuberculosis* (strain H37Rv) appeared to be protective (3); however, many other mycobacterial species have proven to be ineffective. *Mycobacterium dwali*, a strain that is more closely related to *M. leprae* than is BCG as judged by lymphocyte transformation tests (1), was found nearly ineffective (7). Cultures of *Mycobacterium chelonae*, *Mycobacterium diernhoferi*, *Mycobacterium nonchromogenicum*, *Mycobacterium phlei*, *Mycobacterium smegmatis*, and *Mycobacterium vaccae* have recently been found to be inactive against *M. leprae* (C. C. Shepard, unpublished observations). Some of these cultures were selected for their antigenic affinity to *M. leprae* (2). All of these inactive mycobacterial vaccines, including H37Ra, were administered intradermally, the most effective route for BCG and *M. leprae* vaccines; they did not produce the chronic enlargement of the regional lymph node that regularly follows intradermal vaccination with BCG and *M. leprae*. The BCG in the present study was of the Rosenthal strain, but other BCG strains have given similar lymph node enlargement and protection against *M. leprae* infectious challenge. The questions arising from these findings of the

TABLE 1. Results of challenge of vaccinated mice with virulent *M. tuberculosis*

Vaccine	Dose (μg)	No. of mice	S-30 mice ^a	
			No.	%
Before shipment to				
Atlanta				
myc RNA	50	20	15	75
myc RNA	5	30	14	47
myc RNA	0.5	30	7	23
FLA ^b + H ₂ O		30	2	7
H37Ra	100 ^c	30	20	67
Nil		30	8	27
After return from				
Atlanta				
myc RNA	50	20	16	80
myc RNA	5	20	14	70
myc RNA	0.5	20	8	40
H37Ra	100	20	18	90
Nil		20	5	25

^a S-30 mice were mice that survived 30 days.

^b FLA, Freund incomplete adjuvant.

^c A 100- μg portion of H37Ra is equivalent to 5 μg of myc RNA.

distribution of vaccine effectiveness among mycobacterial species remain under study.

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