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

CHARLES C. SHEPARD,* ANNE Y. YOUmans, AND GUY P. YOUmanS

Center for Disease Control, Atlanta, Georgia 30333,* and Department of Microbiology-Immunology, Northwestern University Medical School, Chicago, Illinois 60611

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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 modified 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 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
received H37Ra intraperitoneally in saline suspension in an amount estimated to provide approximately 5 μg 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 μg (wet weight; $10^7$ bacilli, approximately $10^8$ 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 \(10^{-5}\) M. lepra, 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 \(\mu\)g 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. lepra, 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. lepra and M. tuberculosis, of course, differ in antigenic specificity. All mycobacteria share some antigens, but M. lepra 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. lepra 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. lepra infection (4, 7). In contrast, the entire test with the M. tuberculosis challenge is completed within 2 months. Another difference is that the M. lepra 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' 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. lepra 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. lepra. Earlier work, carried out less quantitatively, has provided evidence that R11rv, a strain of M. tuberculosis that is less attenuated than H37Ra, afforded protection against M. lepra 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 duvalii, a strain that is more closely related to M. lepra than is BCG as judged by lymphocyte transformation tests (1), was found nearly ineffective (7). Cultures of Mycobacterium chelonai, Mycobacterium diernhoferi, Mycobacterium nonchromogenicum, Mycobacterium phlei, Mycobacterium smegmatis, and Mycobacterium vaccae have recently been found to be inactive against M. lepra (C. C. Shepard, unpublished observations). Some of these cultures were selected for their antigenic affinity to M. lepra (2). All of these inactive mycobacterial vaccines, including H37Ra, were administered intradermally, the most effective route for BCG and M. lepra vaccines; they did not produce the chronic enlargement of the regional lymph node that regularly follows intradermal vaccination with BCG and M. lepra. The BCG in the present study was of the Renshaw strain, but other BCG strains have given similar lymph node enlargement and protection against M. lepra infectious challenge. The questions arising from these findings of the

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

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Dose ((\mu)g)</th>
<th>No. of mice</th>
<th>S-30 mice* No. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before shipment to Atlanta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>myc RNA</td>
<td>50</td>
<td>20</td>
<td>15 75</td>
</tr>
<tr>
<td>myc RNA</td>
<td>5</td>
<td>30</td>
<td>14 47</td>
</tr>
<tr>
<td>myc RNA</td>
<td>0.5</td>
<td>30</td>
<td>7 23</td>
</tr>
<tr>
<td>FIA* + H₂O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H37Ra</td>
<td>100°</td>
<td>30</td>
<td>20 67</td>
</tr>
<tr>
<td>Nil</td>
<td>30</td>
<td>8 27</td>
<td></td>
</tr>
<tr>
<td>After return from Atlanta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>myc RNA</td>
<td>50</td>
<td>20</td>
<td>16 80</td>
</tr>
<tr>
<td>myc RNA</td>
<td>5</td>
<td>20</td>
<td>14 70</td>
</tr>
<tr>
<td>myc RNA</td>
<td>0.5</td>
<td>20</td>
<td>8 40</td>
</tr>
<tr>
<td>H37Ra</td>
<td>100</td>
<td>20</td>
<td>18 90</td>
</tr>
<tr>
<td>Nil</td>
<td>20</td>
<td>5 25</td>
<td></td>
</tr>
</tbody>
</table>

* S-30 mice were mice that survived 30 days.
* FIA, Freund incomplete adjuvant.
* A 100-\(\mu\)g portion of H37Ra is equivalent to 5 \(\mu\)g of myc RNA.
distribution of vaccine effectiveness among mycobacterial species remain under study.

ACKNOWLEDGMENTS

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LITERATURE CITED