Effect of Rifampin on Immunity to Tuberculosis and on Delayed Hypersensitivity to Purified Protein Derivative

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Mice vaccinated with mycobacterial ribonucleic acid (RNA) produced a high immune response and did not develop delayed hypersensitivity to purified protein derivative (PPD), and rifampin had no effect on the immune response. Mice vaccinated with viable H37Ra cells produced a high immune response and did develop delayed hypersensitivity to PPD. Rifampin had no effect on this immune response, but reduced the footpad reactions to PPD. Both mycobacterial RNA and poly(A:U) served as adjuvants for induction of hypersensitivity to PPD. This hypersensitivity was reduced by the administration of rifampin. Rifampin had no effect on the production of mycobacterial growth inhibitory factor, which is produced following vaccination of mice with mycobacterial RNA or viable H37Ra cells. Rifampin had no effect on the nonspecific phase of the granulomatous response, but did inhibit the secondary allergic phase of this response. The action, therefore, of rifampin that inhibits the induction of delayed hypersensitivity but has no effect on the immune responses against tuberculosis leads to a separation of tuberculin hypersensitivity from cellular immunity to tuberculosis.

Rifampin has been shown to suppress the formation of antibody (1, 11, 13, 19), to be a very effective antimycobacterial drug (10, 19), and to suppress the reaction of purified protein derivative (PPD) in guinea pigs vaccinated with Freund complete adjuvant (3). We have found that rifampin has no effect on the immune response produced in mice vaccinated with our mycobacterial ribonucleic acid (RNA) vaccine (19). Mice and guinea pigs vaccinated with ribosomal or RNA vaccines do not develop tuberculin hypersensitivity (5, 24), nor do their lymphocytes produce migration inhibitory factor when stimulated with PPD. The results of these studies indicate that delayed hypersensitivity to PPD and cellular immunity to tuberculosis are dissociable entities. Further experiments using rifampin, as will be seen, support this conclusion.

MATERIALS AND METHODS

Animal experiments. Male inbred C57Bl/6 mice were obtained from the Jackson Laboratory, Bar Harbor, Me. When used, they weighed between 18 to 22 g and were housed 10 to a cage. The mice were vaccinated intraperitoneally, held for 4 weeks, and then challenged intravenously with 1.0 mg of the highly virulent strain H37Rv of Mycobacterium tuberculosis (21). Survival time was measured by a daily inspection of the cages, and the time of death of each mouse was recorded. After 30 days, the surviving mice were killed, since by that time most or all of the control, nonvaccinated mice were dead, and the percentage of survival of each group was determined. The reliability and validity of the above procedures for measurement of the immune response of mice has been published previously (21).

Vaccines. The mycobacterial RNA was prepared by the method we have previously described (14, 17) and was used within 3 days after preparation. Just before vaccination, it was incorporated into Freund incomplete adjuvant (18). The viable cells from which the mycobacterial RNA vaccine was prepared were of the attenuated strain, H37Ra, of M. tuberculosis. These cells were used, in addition, as a control for the mycobacterial RNA vaccine. The manner in which we maintain and grow these cells has been described by us in detail (16). Other vaccines consisted of mycobacterial RNA (50 μg/mouse) mixed with PPD (25 μg/mouse) and poly(A:U) (300 μg/mouse) mixed with PPD (25 μg/mouse). These vaccines also were incorporated into Freund incomplete adjuvant just before vaccination.

Compounds. Poly(A:U) was obtained from Miles Laboratories, Elkhard, Ind.; PPD was furnished by the National Institute of Allergy and Infectious Diseases, Bethesda, Md. (lot PH43-68-1742); rifampin was purchased from Calbiochem, San Diego, Calif. This compound was administered orally in the drinking water, approximately 400 μg/mouse per day. Rifampin treatment was begun 2 days before vaccination and stopped 2 to 3 days before challenge, since it is a potent inhibitor of the growth of the virulent strain, H37Rv (10, 19).

Footpad tests. Five to 10 mice of each experimental group were injected with 2 μg of PPD contained in 0.05 ml of diluent into the right rear foot-
pad, using a 30-gauge needle. The same volume of the diluent was injected into the left rear footpad and acted as a control. The thickness of the footpad was measured at 4, 24, 48, and 72 h using a Schnelltaster caliper (H.C. Drop-Schnell GmbH, Postfach, Germany).

Preparation of mycobacterial growth inhibitory factor. The mycobacterial growth inhibitory factor was prepared from spleen cells isolated from mice vaccinated with viable cells of the H37Ra strain of M. tuberculosis. The technical details have been given in previous publications (4, 6-8, 12).

Pulmonary granulomatous response. Adult mice were injected intravenously with 5.0 mg of viable H37Ra cells suspended in 0.2 ml of diluent. At appropriate times thereafter, five mice were killed by injecting sodium pentobarbital, and the lungs were removed and weighed. Five mice that had not received H37Ra cells were sacrificed also at each time, and their lungs were removed and weighed. We have shown in a previous publication that the lung weight after intravenous injection of H37Ra cells is a reflection of the degree of the pulmonary granulomatous response (22). A granulomatous index can be calculated by dividing the mean weight of the lungs of the injected mice by the mean weight of the lungs of the noninjected control mice (22).

RESULTS

The effect of rifampin on the immune response obtained in mice vaccinated with mycobacterial RNA and viable H37Ra cells is shown in Table 1. Mice vaccinated with mycobacterial RNA were protected against tuberculosis and had negative footpad reactions to PPD. Treatment with rifampin had no effect on the immune response. Mice vaccinated with the viable H37Ra cells also were protected against tuberculosis, and these mice had strong footpad reactions to PPD. These reactions were significantly reduced in the group of mice treated with rifampin. These results show clearly that rifampin had no effect on the immune response to RNA, but does have an effect on the development of delayed hypersensitivity to PPD. They, therefore, lend further support to the thesis that immunity to tuberculosis and tuberculin hypersensitivity are independent phenomena.

If mice were vaccinated with viable H37Ra cells and were treated with rifampin it was found, from these and other experiments, that the viable H37Ra cells were not uniformly immunogenic in mice. The 0.5-μg vaccinating dose was not as immunogenic in the treated mice (Table 1). In other experiments both the 5.0- and the 50.0-μg vaccinating doses produced a lower response. This effect varied between experiments; apparently the rifampin was not acting uniformly. Since treatment with rifampin was started 2 days before vaccination, and since it is a potent inhibitor of the growth of tubercle bacilli (10, 19), it was possible that the drug was killing the viable H37Ra cells before they had time to induce a maximum immune response against tuberculosis. The immune responses obtained in these experiments, in fact, are similar to those we have obtained in mice vaccinated with heat-killed cells (23). To test this theory, mice were treated with rifampin 2 days before vaccination, and treatment of another vaccinated group was not started until 10 days after vaccination. The results of these experiments are given in Table 2. Mycobacterial RNA again was highly immunogenic in both vaccination doses in mice, whether the mice were treated with rifampin or not. The viable H37Ra cells were also highly immunogenic if

<table>
<thead>
<tr>
<th>Immunizing prep</th>
<th>Treated with rifampin</th>
<th>Amt of RNA (μg)</th>
<th>No. of mice</th>
<th>No. of S-30 mice</th>
<th>% of S-30 mice</th>
<th>Footpad reaction (mm)</th>
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<td>57</td>
<td>56</td>
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<td></td>
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<td>19</td>
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<td>0.04</td>
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<td>55</td>
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<tr>
<td></td>
<td></td>
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<td>20</td>
<td>15</td>
<td>75</td>
<td>ND*</td>
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<td>86</td>
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<td>50.0</td>
<td>57</td>
<td>12</td>
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<td>12</td>
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<tr>
<td>Controls</td>
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<td>57</td>
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<td>21</td>
<td>0.05</td>
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</tr>
</tbody>
</table>

* S-30, Number of mice that survived >30 days.
* ND, Not done.
* 50 μg of RNA = approximately 1 mg (moist weight).
untreated with rifampin. However, the H37Ra-vaccinated mice in which treatment was started 2 days before vacinnation had a lower or no immune response, but if the treatment was delayed until 10 days after vaccination the rifampin had no effect on the immune response. This suggests that the H37Ra cells had sufficient time to be phagocytized, liberate their RNA, and immunize against tuberculosis. Even though the rifampin was started 10 days after vaccination, it still significantly reduced tuberculin hypersensitivity.

Since mycobacterial RNA has double-stranded characteristics (18), mice were injected intraperitoneally with mycobacterial RNA that had been mixed with PPD to determine whether the adjuvant action of mycobacterial RNA (2, 25) might produce a footpad reaction to PPD. PPD injected alone does not induce tuberculin hypersensitivity. The results of these experiments are shown in Table 3. The mice vaccinated with a mycobacterial RNA-PPD mixture developed tuberculin hypersensitivity, as indicated by the strong footpad reactions. The induction of this hypersensitivity, however, was completely suppressed by treatment with rifampin, but the immunogenic activity of the mycobacterial RNA again was not affected by treatment with rifampin, whether PPD was given or not.

The synthetic double-stranded polyribonucleotide, poly(A:U), was substituted for mycobacterial RNA and mixed with PPD (2) to determine whether it would act as an adjuvant for PPD to produce a positive footpad reaction to PPD. The mice vaccinated with poly(A:U) mixed with PPD did develop positive footpads, and these were reduced in a similar group of vaccinated mice treated with rifampin (Table 3).

Experiments also were done to test the effect of rifampin on the production of mycobacterial growth inhibitory factor. This is a lymphocyte product which will inhibit the intracellular growth of virulent tubercle bacilli (4, 6-8, 12). Table 4 shows results which indicate that rifampin did not inhibit the formation of mycobacterial growth inhibitory factor; in fact, it seemed to increase the amount of inhibition. These findings suggest again that cellular immunity and the production of tuberculin hypersensitivity are not related.

Finally, the effect of rifampin on the pulmonary granulomatous response in mice induced by the intravenous injection of 5.0 mg of viable H37Ra cells was determined, and this is shown in Fig. 1. Rifampin had no effect on the nonspecific rise of the granulomatous response that occurred at day 2, but it did inhibit the specific allergic granulomatous response to the H37Ra cells, which began approximately 23 days after vaccination. At day 23 there was a slight rise in the lung weights of the treated

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**Table 2. Effect of rifampin given 2 days before and 10 days after vaccination on the immune response and footpad reaction**

<table>
<thead>
<tr>
<th>Immunizing prepn</th>
<th>Amt of rifampin given (µg)</th>
<th>Time drug given (days)</th>
<th>Amt of RNA injected (µg)</th>
<th>No. of mice</th>
<th>% of S-30 mice</th>
<th>Footpad reaction (mm)</th>
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<tr>
<td>Mycobacterial RNA</td>
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<td>50.0</td>
<td>20</td>
<td>95</td>
<td>0.02</td>
<td></td>
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<tr>
<td>Mycobacterial RNA</td>
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<td>5.0</td>
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</tr>
<tr>
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<td>50.0</td>
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<td>100</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>H37Ra cells</td>
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<td>5.0</td>
<td>10</td>
<td>90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a 50.0 µg of RNA = approximately 1.0 mg (moist weight).

P = <0.001.

c Given in the drinking water.
mice, which correlated with the high rise in the lung weights from the nontreated mice; however, by day 30 the lung weights in the treated mice were again reduced. At day 30 treatment with rifampin was stopped, and the remaining mice were held for approximately 4 weeks to determine whether during this time the lungs of the formerly treated mice would eventually increase in size and be comparable to the nontreated mice. Weights of the lungs of the treated mice increased slightly by day 56, but then began decreasing, as did the lungs from the nontreated mice, so that by day 62 the weights of the lungs of the two groups were almost identical (Fig. 1).

Shown in Table 5 is a summary of our results comparing the effect of rifampin on the immune response to mycobacterial RNA and viable H37Ra cells, on delayed hypersensitivity, on mycobacterial growth inhibitory factor production, and on the granulomatous response. We found that (i) mice vaccinated with mycobacterial RNA produced a high immune response and did not develop delayed hypersensitivity to PPD, and rifampin had no effect on the immune response. (ii) Mice vaccinated with viable H37Ra cells produced a high immune response and did develop delayed hypersensitivity to PPD. Rifampin had no effect on this immune response, but it reduced the footpad reactions to PPD. (iii) Both mycobacterial RNA and poly(A:U) served as adjuvants for induction of hypersensitivity to PPD. This hypersensitivity was reduced by the administration of rifampin. (iv) Rifampin had no effect on the production of mycobacterial growth inhibitory factor, which is produced after vaccination of mice with RNA or H37Ra cells. (v) Finally, rifampin had no effect on the nonspecific phase of the granulomatous response, but it did inhibit the secondary allergic phase of this response.

Therefore, the action of rifampin, which inhibits the induction of delayed hypersensitivity but has no effect on the immune responses against tuberculosis, leads to a separation of tuberculin hypersensitivity from cellular immunity to tuberculosis.

**DISCUSSION**

The differential effect of rifampin on the induction of immunity to tuberculosis and on the induction of tuberculin hypersensitivity is difficult to explain if, as is commonly believed, both are T lymphocyte-mediated phenomena. There is some reason to think that different T lymphocyte populations may be involved in the two phenomena (9, 20). If this should be the case it is conceivable that rifampin might inhibit deoxyribonucleic acid-dependent RNA polymerase activity in those T lymphocytes responsible for delayed-type hypersensitivity, but be unable to do so, for unknown reasons, in those T lymphocytes responsible for immunity to infection. On the other hand, it is possible that rifampin in both cases does not act upon the cells involved in the efferent limb. The manifestations of the two immune responses in vivo are very different. Tuberculin hypersensitivity usually appears as an acute necrotizing inflammatory reaction involving death and disintegration of macrophages and other tissue cells. Cellular immunity to tuberculosis, on the other hand, is characterized by the inhibition of multiplication of virulent tubercle bacilli within macrophages. Cell death (necrosis) is not a
Regardless of the mechanism involved the results obtained in this study with rifampin clearly support the thesis that tuberculin hypersensitivity and immunity to tuberculosis are independent phenomena.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


