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Immunological Responsiveness of Tuberculosis Patients Receiving Rifampin

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Rifampin has been shown to impair both humoral and cell-mediated immune responses in animal models. In order to detect a similar effect in man, 11 patients with active tuberculosis were evaluated before, 2 weeks after, and 12 to 16 weeks after initiating rifampin. Several parameters were serially measured including blood lymphocytes, intradermal response to intermediate strength tuberculin (PPD), and in vitro proliferative responses to phytohemagglutinin (PHA), a nonspecific mitogen, and to the specific antigens, PPD and influenza A. No changes in lymphocyte counts were noted. No changes in response were noted 2 weeks after beginning treatment with rifampin. However, compared with initial and 2-week responses, the PHA response was reduced by 47%, the PPD by 68%, and the influenza by 75%, and 6 of 11 patients showed no induration after tuberculin skin testing at the 12- to 16-week point. These results indicate that in doses employed for the treatment of tuberculosis, rifampin has an immunosuppressive effect in man that develops gradually.

Rifampin is currently recognized as one of the most effective new agents for tuberculosis therapy (8, 13). In addition to its antibacterial properties, rifampin appears to have immunosuppressive activity. Paunescu (10) and others (3), using animal models, found that rifampin impairs antibody responses and skin test reactivity to several antigens. This effect is dose related and promptly reverses when the drug is discontinued. Rabbits treated with this agent show prolonged survival of skin grafts (12).

In vitro studies demonstrate that rifampin reduces the proliferative responses of human lymphocytes to both tuberculin (PPD) and phytohemagglutinin (PHA) (2, 9) in a dose-dependent manner. Rifampin cream applied to smallpox vaccination sites impairs production of antivaccinia hemagglutination-inhibiting antibody (6). In patients receiving rifampin orally, humoral antibody responses to keyhole limpet hemocyanin are decreased (4).

In the present study parameters of immune function in tuberculosis patients treated with rifampin were sequentially measured. Prolonged therapy was associated with decreased skin test reactivity to PPD and suppression of in vitro lymphoproliferative responses to both specific antigens and a nonspecific mitogen, PHA.

MATERIALS AND METHODS

Patients. Eleven adults of both sexes, aged 23 to 68 years (average 48 years) with active pulmonary tuberculosis, who were either hospitalized or being managed in the County Tuberculosis Clinic, were studied. Prior to initiating rifampin therapy, all patients were receiving two or more antituberculous drugs including isoniazid, streptomycin, and ethambutol. They had been treated with these antituberculosis drugs for periods ranging from days to several months. No patients had other major underlying diseases, and none was receiving corticosteroids or cytotoxic agents.

Rifampin dosage. Following the initial immunologic survey, treatment of all patients with 600 mg of rifampin in a single, daily morning dose was begun. Other antituberculosis drugs were continued throughout the study period.

Blood specimens. Blood was collected from each patient at three specific time intervals: before, 2 to 3 weeks after, and 12 to 16 weeks after initiation of rifampin therapy. Blood samples were obtained approximately 2 h after the patient had received his daily medication; serum was stored at −20 C. For lymphocyte studies, 30 to 50 ml of blood was collected in heparin (0.25 mg of a 1% heparin solution per 5 ml of blood) and handled as indicated below.

Vaccine studies. Patients received 0.5 ml of a 1972-73 formula, bivalent, influenza vaccine containing 700 CCA A/Aichi/68 and 300 CCA B/Mass/71 viruses. Hemagglutination-inhibiting (HAI) antibodies to influenza A/Aichi were measured as previously described (11).

Lymphocyte cultures. Samples of heparinized blood were processed within 2 h of collection by using assay techniques reported in detail elsewhere (15). Peripheral blood mononuclear cells were separated by the Ficoll-Hypaque method. Each culture contained 3.0×10^6 cells; antigen-stimulated cultures contained either PPD (20 μg/culture), PHA (0.1 ml/culture), or influenza A antigen (0.1 ml of a suspension containing 800 hemagglutinating units of inactivated influenza A/Aichi/68 virus, the vaccine strain). Antigen-stimulated and control cultures were incubated for 5 days; the cultures containing PHA and unstimulated controls were incubated for 3 days. At each time point, at least three cultures were incubated with antigen, and two or three unstimulated cultures served as controls.

Four hours prior to termination of the incubation, 1.0 μCi of tritiated thymidine was added to each culture. Each sample was counted in a Tricarb liquid scintillation counter; results were expressed as counts per minute per 10^6 cells. Values in replicate cultures were averaged to determine the mean isotope uptake. In addition, for each patient, a stimulation ratio was calculated by dividing the mean uptake in antigen-stimulated cultures by that in unstimulated replicates. The average stimulation ratio for most series was determined by $\log_{10}$ transformation of individual values because this served to normalize the distribution. Differences between series were analyzed with the Mann-Whitney U Test.

Other studies. White blood cell counts with differential cell counts were also performed at intervals by routine techniques.

Skin tests with Tween-stabilized intermediate strength tuberculin were done at intervals by the Mantoux technique.

RESULTS

Tuberculin skin tests. Prior to initiating rifampin therapy, all 11 patients showed positive reaction to intermediate strength tuberculin. The average diameter of induration was 19.6 ± 1.3 mm (SE). At 2 weeks, 10 of 11 remained reactive; the mean diameter was 18.1 ± 3.0 mm. However, after 12 to 16 weeks of therapy, six previously reactive patients had converted to nonreactivity. The remaining five showed positive responses which were unchanged from the initial tests. Thus, prolonged therapy inhibited cutaneous responses to tuberculin in a portion of the treated patients.

Peripheral blood lymphocyte count. Pretreatment, 2-week, and 12- to 16-week blood lymphocyte counts were not significantly different (Fig. 1). The values at these three times were 2,064/mm³ ± 772, 2,432 ± 511, and 2,136 ± 417, respectively.

Lymphocyte cultures. In each patient, lymphoproliferative responses to the nonspecific mitogen, PHA, and two specific antigens were measured at the three time periods. There was no significant change in the response to PHA at 2 weeks. However, after 12 to 16 weeks, the mean isotope uptake was reduced by 47% (Fig. 2).

Responses to PPD are expressed as a stimulation ratio (Fig. 3). The mean value for PPD-

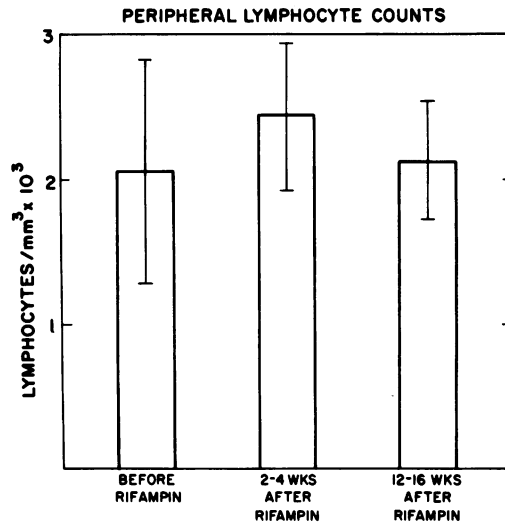

FIG. 1. *Peripheral blood lymphocyte counts in relation to the initiation of rifampin therapy. Mean ± SE.*

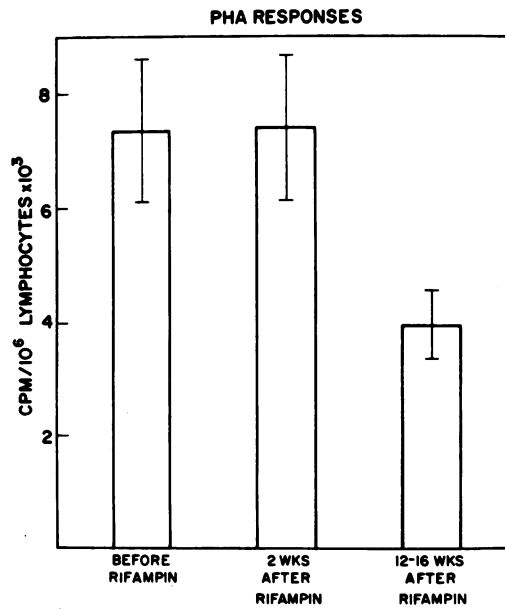

FIG. 2. *In vitro lymphocyte responses to phytohemagglutinin (PHA) in relation to the initiation of rifampin therapy. The value obtained after 12 to 16 weeks of rifampin was significantly lower ($P < 0.025$).*

stimulated lymphocytes in the pretreatment specimen (7.9 ± 1.1) was not significantly different from that at 2 weeks (7.0 ± 0.9). However, at 12 to 16 weeks, the ratios were significantly lower (mean 2.5 ± 0.8). It should be noted that the activity in the unstimulated control cultures was not altered at any time; thus, reduction in stimulation ratios represented a true depression of antigen-induced cell replication.

Similar responses were observed in influenza-stimulated cultures (Fig. 4). The average stimulation ratios in the pretreatment and 2-week samples (6.8 ± 1.7 and 5.9 ± 1.3, respectively) were significantly greater than in the 12- to 16-week samples (1.7 ± 0.7). Thus, responses to PPD and influenza A were reduced by 68 and 75%, respectively.

Responses to influenza vaccine. Nine persons received a single dose of influenza vaccine 2 weeks after initiating rifampin therapy. HAI antibody responses were measured 12 to 28 days after this dose. Six of the nine persons had fourfold or greater rises in HAI antibodies against the vaccine strain of Influenza A (Fig. 5). The three who failed to develop significant increases had reciprocal HAI titers of 256, 1,024, and 4,096 at the time vaccine was given; such

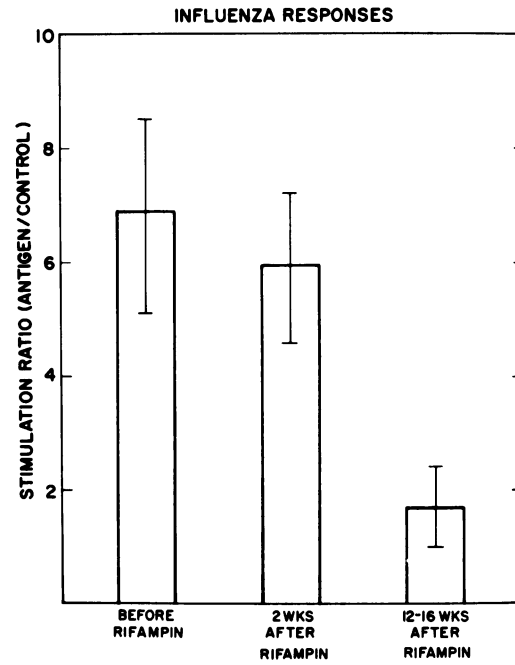

FIG. 4. *In vitro lymphocyte responses to influenza A antigen in relation to the initiation of rifampin therapy. The value obtained after 12 to 16 weeks of rifampin was significantly lower ($P < 0.025$).*

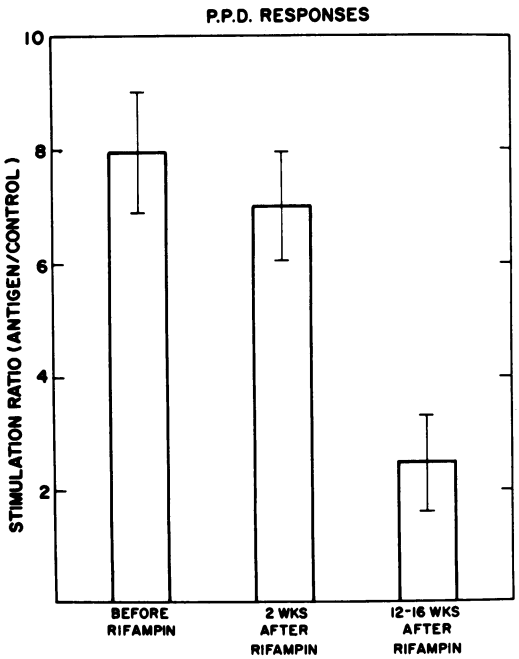

FIG. 3. *In vitro lymphocyte responses to tuberculin (PPD) in relation to the initiation of rifampin therapy. The value obtained after 12 to 16 weeks of rifampin was significantly lower ($P < 0.025$).*

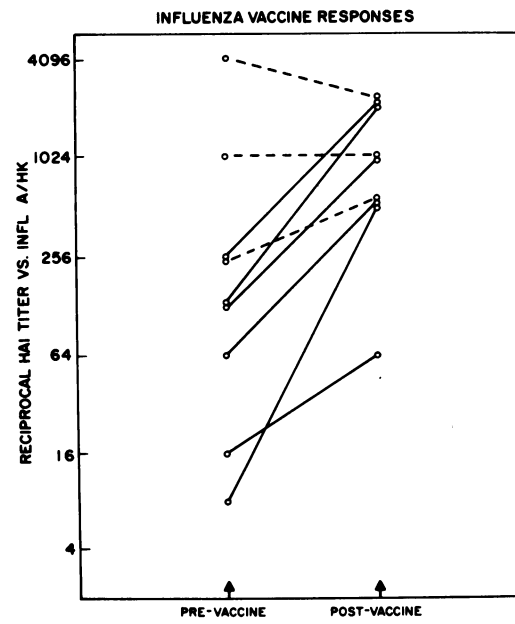

FIG. 5. *Serum hemagglutination-inhibiting antibody responses to influenza A before and after influenza vaccine administration. Vaccine was administered after 2 weeks of therapy.*

patients are not likely to show rises in antibody titer after vaccination (5).

Studies in nontuberculosis subjects. To test the effect of rifampin on the in vitro responses of lymphocytes to PHA, cultures were established from six normal subjects. At doses in excess of 10 μg/ml, rifampin impaired proliferative responses (Fig. 6). Lesser quantities did not inhibit proliferation; in fact, in lower amounts this drug may have a stimulatory effect. The isotope incorporation in PHA-stimulated cultures containing 0.1 μg/ml was appreciably greater than in those cultures established without rifampin. Rifampin itself was not stimulatory. Cultures containing the drug in concentrations of 0.25 to 125 μg/ml showed no increased isotope incorporation as compared with unstimulated replicates.

DISCUSSION

This investigation indicates that the antituberculous drug, rifampin, impairs cell-mediated immune responses in humans. The immunosuppressive effect develops gradually. There was no change after 2 weeks of therapy in either in vitro lymphoproliferative responses to the nonspecific mitogen, PHA, or to the specific antigens, PPD and influenza A, or in skin test reactivity to tuberculin. After 12 to 16 weeks, there was a decrease in lymphoproliferative responses to both specific and nonspecific stimuli, and 6 of 11 patients converted their tuberculin skin test from positive to negative.

In the present study, the effect of rifampin on humoral antibody production was tested at 2 to 4 weeks after starting rifampin. This was at a time when no changes in lymphoproliferation were evident. The antibody titers did not indicate any suppression. These responses to influenza A may be considered anamnestic type because all persons showed antibodies prior to immunization. We have not tested either primary or anamnestic type responses at 12 to 16 weeks, a time when the immunosuppressive effect of rifampin is evident, although such studies are planned.

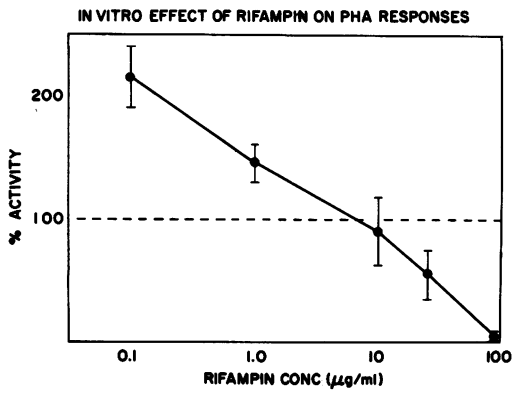

FIG. 6. *In vitro lymphocyte responses to PHA in the presence of graded doses of rifampin.*

As shown by these and other studies, rifampin, when added to lymphocyte cultures, can inhibit proliferative responses to PHA (2, 9). It should be noted that such inhibition is not observed until the in vitro concentration exceeds 10 μg/ml, an amount greater than the usual blood levels (14).

These results indicate that prolonged therapy with rifampin in humans exerts an immunosuppressive effect and are in accord with previously reported studies in animals (3, 10, 12). In several animal models, immunosuppression has been observed promptly after institution of treatment and was rapidly reversed when the drug was withdrawn. The delayed suppression of cellular immunity observed in this clinical study differs from what has been observed in animals; the explanation for this delayed effect is not known.

Recently, two studies have been reported that are in agreement with the results of the present study. Bassi et al. (1) failed to show any evidence of immunosuppression in human volunteers given cholera vaccine and maintained on rifampin for only 16 days. Mukerjee et al. (7) performed tuberculin skin tests in adult tuberculous patients receiving rifampin and showed a suppression of the response.

The mechanisms by which rifampin exerts its immunosuppressive activities are unclear. The drug does not produce measurable changes in blood lymphocyte counts. Although these counts may not accurately reflect the total body pools of lymphocytes, their lack of depression suggests that the immunosuppressive activity is not due to depletion of immunologically competent cells. Considering the effect of rifampin in bacterial systems, it may be that this drug impairs certain functions of immunologically competent cells, limiting their ability to participate in cellular responses.

Although rifampin exerted a demonstrable immunosuppressive effect, all patients showed favorable responses to therapy. Acid-fast bacilli were eliminated from the sputum of all patients, and all are currently being treated as outpatients.

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LITERATURE CITED

1. Bassi, L., L. DiBernardino, V. Arioli, L. G. Silvestri, and E. L. Chérié Lignière. 1973. Conditions for immunosuppression by rifampicin. J. Infect. Dis. **128**:736-744.
2. Dajani, B. M., M. S. Canady, J. S. Thompson, and J. E. Kasik. 1972. Rifampicin: an immunosuppressant? Lancet **ii**:1094.
3. Dajani, B. M., J. E. Kasik, and J. S. Thompson. 1973. Effect of rifampin on the immune response in guinea pigs. Antimicrob. Ag. Chemother. **3**:451-455.
4. Graber, C. D., J. Jebaily, R. L. Galphin, and E. Doering. 1973. Light chain proteinuria and humoral immunoincompetence in tuberculosis patients treated with rifampin. Amer. Rev. Resp. Dis. **107**:713-717.
5. Kilbourne, E. D., W. T. Butler, and R. D. Rossen. 1973. Specific immunity in influenza—summary of influenza workshop III. J. Infect. Dis. **127**:220-236.
6. Moshkowitz, A., N. Goldblum, and E. Heller. 1971. Studies on the antiviral effect of rifampicin in volunteers. Nature (London) **229**:422-424.
7. Mukurjee, P., S. Schuldt, and J. E. Kasik. 1973. Effect of rifampicin on cutaneous hypersensitivity to purified protein derivative in humans. Antimicrob. Ag. Chemo. **4**:607-611.
8. Newman, R., B. Doster, F. J. Murray, and S. Ferebee. 1971. Rifampin in initial treatment of pulmonary tuberculosis. Amer. Rev. Resp. Dis. **103**:461-476.
9. Nilsson, B. S. 1971. Rifampicin: an immunosuppressant? Lancet **ii**:374.
10. Păunescu, E. 1970. *In vivo* and *in vitro* suppression of humoral and cellular immunological responses by rifampicin. Nature (London) **228**:1188-1190.
11. Ruben, F. L., and G. G. Jackson. 1972. A new subunit influenza vaccine: acceptability compared with standard vaccines and effect of dose on antigenicity. J. Infect. Dis. **125**:656-664.
12. Serrou, B., C. Solassol, H. Joyeux, H. Pujol, and C. Romieu. 1972. Immunosuppressive effect of rifampicin. Transplantation **14**:654-655.
13. Vall-Spinosa, A., W. Lester, T. Moulding, P. T. Davidson, and J. K. McClatchy. 1970. Rifampin in the treatment of drug-resistant *mycobacterium tuberculosis* infections. New Engl. J. Med. **283**:616-621.
14. Verbist, L., and A. Gyselen. 1968. Antituberculous activity of rifampin *in vitro* and *in vivo* and concentrations attained in human blood. Amer. Rev. Resp. Dis. **98**:923-932.
15. Winkelstein, A., F. L. Ruben, S. F. Tolchin, B. H. Pollock. 1974. Mechanisms of immunosuppression: effect of cyclophosphamide on responses to influenza immunization. J. Lab. Clin. Med. **83**:504-510.