Repeated Tuberculin Testing in Patients
With Active Pulmonary Tuberculosis*

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Objective: The proportion of tuberculin reactors in a population and the intensity of tuberculin reactions have been shown to increase with increasing exposure to mycobacterial infection, eg, repeated BCG immunization. These observations suggested that tuberculin reactivity would become uniformly high in individuals with a high mycobacterial load who did not have a known cause of anergy. Since tuberculin reactivity has been measured to evaluate the possible genetic regulation of responses to mycobacteria in humans, it is important to study its behavior under conditions of ongoing, maximal exposure to mycobacteria. In the present study, we determined the mean size of tuberculin reactivity in BCG-immunized and unimmunized patients with pulmonary tuberculosis of recent onset, and the stability of tuberculin reactions during and after treatment of pulmonary tuberculosis.

Method: Serial tuberculin testing was performed on patients with newly diagnosed active pulmonary tuberculosis diagnosed over a period of 2 years at the National Institute for Respiratory Diseases in Santiago, Chile. The first tuberculin test was performed at the time of diagnosis in 58 patients. Repeated tuberculin testing was performed 2 weeks later in 15 patients with initial reaction sizes <15 mm. Four additional tuberculin tests were performed, one each at 3-months intervals in 42 patients regardless of the size of the initial tuberculin reaction.

The proportion of tuberculin reactors in a population and the mean size of tuberculin reactions have been shown to increase with increasing exposure to mycobacterial infection. Bacillus Calmette-Guerin (BCG) immunization offers an opportunity to observe the development of tuberculin reactivity after a known mycobacterial exposure. Unimmunized, young children uninfected with mycobacteria are tuberculin negative. BCG vaccination causes the development of tuberculin reactivity in varying percentages of individuals. The percentage of reactive individuals and the intensity of tuberculin reactivity increases further on repeated BCG immunization, but without reaching uniformity for all individuals with the same number of BCG scars. These differences may be attributed to varying effectiveness of BCG vaccines, including the number of viable mycobacteria, and to the time lapsed since immunization. Patients with tuberculosis offer an opportunity to study tuberculin reactivity elicited by an ongoing exposure to a high mycobacterial load. Tuberculin reactivity has been measured to evaluate the possible genetic regulation of responses to mycobacteria in humans. In the general population, however, the exposure to subclinical mycobacterial infection is difficult to evaluate, and the stability of tuberculin reactivity in patients with tuberculosis has not been determined. Thus, associations between genetic markers and one-time tuberculin reactivity may be significantly influenced by transient, nongenetic factors, including the effect of prior BCG immunization. In the present study, we determined the influence of prior BCG immunization on the intensity of tuberculin reactivity in patients with pulmonary tuberculosis who did not have concomitant debilitating diseases, as well as the stability of tuberculin reactions.

Results: Tuberculin reactions at entry had a unimodal distribution in patients both with and without BCG scars (14.8±5.0 mm and 16.5±5.2 mm, respectively). A second tuberculin test in patients with initial reaction sizes <15 mm showed a moderate, statistically significant increase in the mean reaction size (PPD1: 10.1±3.2 mm; PPD2: 11.9±4.8 mm). Repeated tuberculin testing over 1 year revealed no significant changes in reaction size. The mean reaction sizes were 15.8±5.0 mm at entry, 15.5±5.4 mm at 3 months, 17.2±5.2 mm at 6 months, 17.0±5.1 mm at 9 months, and 16.7±5.4 mm at 12 months. The standard deviation of a random observation within patients was 5.3 mm. The largest variations due to increased reactivity after 6 months of treatment were observed in patients with reaction <15 mm at entry compared with hyperergic patients, and in BCG-immunized patients compared to unimmunized patients.

Conclusions: In the presence of an ongoing mycobacterial infection, patients without anergizing conditions express a tuberculin reactivity that is relatively constant during and after treatment of pulmonary tuberculosis. The size and stability of the reactions seem to be determined by individual conditions that include the tuberculin reactivity at the time of diagnosis and the BCG immunization status.

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during and after completing a treatment regimen that lasted 6 months.

**Material and Methods**

The study population consisted of 58 patients between 14 and 65 years of age with recently diagnosed pulmonary tuberculosis who were seen at the National Institute for Respiratory Diseases and Thoracic Surgery in Santiago, Chile. All patients had abnormal chest roentgenograms fulfilling the criteria for pulmonary, class 3 tuberculosis, and at least one positive sputum smear for acid-fast bacilli (AFB). All positive smears were confirmed by culture in Loewenstein-Jensen medium. None of the patients received treatment before entry. All eligible patients whose conditions were diagnosed over a 2-year period who consented to participate were entered into the study. None of these patients had extrapulmonary tuberculosis or known causes of anergy, eg, severe malnutrition, chronic hepatic or renal disorders, or corticosteroid treatment. Human immunodeficiency virus (HIV) testing was not performed in this patient population. However, an HIV serology study performed 1 year later on 1,159 patients with active tuberculosis in Santiago, Chile, including the area of our study, revealed only four patients who were HIV-positive.

Treatment during the initial 29 days consisted of streptomycin, 0.75 g/d intramuscularly (IM), isoniazid, 300 mg/d orally, rifampin, 600 mg/d orally, and pyrazinamide, 2 g/d orally. Then, treatment was continued for a total of 6 months with isoniazid, 800 mg twice weekly (50 doses) and rifampin, 600 mg/d orally twice weekly (50 doses) (1SHRZ6 H1R6). Recurrence rate with this treatment is 0.7 percent at 15 months and less than 3 percent at 5 years.

Of the 58 patients entered into the study, 42 were followed up in the same clinic over the subsequent 12 months. Sixteen patients were transferred to their original referring medical centers and were unavailable for follow-up for our study. These patients did not differ in their age and sex distribution and in their response to treatment from those who remained in the study. The conditions of all patients transferred to other centers improved, as defined by persistent negative sputum smears and cultures, and by regression of pulmonary lesions detected by chest roentgenograms. Of the 42 patients we followed up directly over 1 year, 41 had improved conditions at the end of the treatment period. One patient died as a consequence of a relapse of his pulmonary tuberculosis 12 months after entering the study. A check of the National Registry of HIV-infected individuals in Chile, where all patients with tuberculosis have been tested for HIV antibodies since 1989, revealed that none of the 57 surviving patients had relapsed or had been found to be HIV seropositive.

The BCG immunization status of each patient was determined by recording the presence of BCG scars from immunizations at birth and at 6 or 14 years of age. Tuberculin testing was performed by two trained nurses using purified protein derivative (PPD) tuberculin RT23 obtained from the Statens Serum Institute in Copenhagen, Denmark, and diluted in polysorbate 80 (Tween 80). Two units of PPD tuberculosis RT23 is equivalent to 5TU of PPD-5. One-tenth of 1 ml of tuberculin solution was injected intradermally into the volar surface of the forearm, using glass PPD syringes (Omega) with disposable No. 27 needles. The size of the induration was read in millimeters as the transverse diameter of induration after 72 h. Reproducibility of skin test reading was tested in patients with tuberculosis prior to this study. Variability between blind readings of the same tuberculin reactions by the nurses performing the study was 0.8 ± 1 mm. Induration ≥10 mm was considered to be positive; induration <10 mm was defined as negative. Positive reactions with indurations ≥15 mm were defined as hyperergic.

All patients were tested by placing PPD 1 on the upper half of the left forearm before or simultaneously with the beginning of treatment. A second tuberculin test (PPD 2) was applied 2 weeks later on the opposite forearm to 15 patients who were not hyperergic when tested at entry. Additional tuberculin tests were applied at 3, 6, 9, and 12 months in all available patients, regardless of the size of their PPD reaction at entry. These sequential tuberculin tests were applied by alternating the forearm used and placing each additional PPD approximately 5 cm distal to the previous one. Results were read without knowledge of the results of the preceding test(s).

The difference between tuberculin reactivity among patients with and without BCG scar(s) at entry was analyzed using Student's t test for unpaired samples. Results were confirmed using the nonparametric Kruskall-Wallis test. The significance of changes in tuberculin reaction size between PPD1 and PPD2 was assessed by the two-tailed paired Student's t test and by the Wilcoxon signed-rank test. The comparison of tuberculin reactivity among and within patients at different times after diagnosis was performed using one-factor analysis of variance (ANOVA) for repeated measures.

**Results**

At entry, 35 patients did not have BCG scars, 18 patients had one BCG scar, and 5 patients had two BCG scars. The mean (±SD) age was 41 ± 14 years for patients without BCG scars and 26 ± 4.4 years for patients with BCG scar(s). Sixty percent of patients were men and 40 percent were women. Sex distribution in the immunized and unimmunized groups was similar.

The mean tuberculin reaction size of the first tuberculin test (PPD1) was 16.5 ± 5.2 mm for 35 patients without BCG scars and 14.8 ± 5.0 mm for 23 patients with BCG scar(s). This small difference was not significant. Of the 35 patients without a BCG scar, 1 had a negative reaction, 9 had reactions ≥10 and <15 mm, and 25 were hyperergic (Fig 1). Of 23 patients with one BCG scar, 4 were negative, 4 were positive, and 15 were hyperergic. All five patients with two BCG scars were hyperergic. Of the 58 patients in our study, a total of 5 (8.6 percent) had negative reactions at the time of diagnosis.

Short-term changes in tuberculin reactivity were tested by performing PPD2 2 weeks later in 15 of the

![Figure 1. Histograms of the tuberculin reaction at the time of diagnosis in 58 patients with pulmonary tuberculosis with and without BCG scars.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21665/ on 04/12/2017)
18 nonhypersergic patients. In this group, the size of the reaction to PPD1 was 10.1 ± 3.2 mm, and the size of the reaction to PPD2 was 11.9 ± 4.8 mm (p < 0.04). In two patients, reactions to PPD2 were smaller than to PPD1. In all other patients, the reaction was identical or larger. The maximum increase was 7 mm. Of four patients with PPD1 reactions <10 mm, two patients remained negative, with increases from 4 to 5 mm, and two patients became positive. One of these patients increased from 7 to 10 mm, and the other patient increased from 9 to 12 mm on retesting with PPD2.

Forty-two patients were followed up during the 1-year study period. The mean tuberculin reaction size at entry for this group was 15.8 ± 5.0 mm; at 3 months it was 15.5 ± 5.4 mm; at 6 months it was 17.2 ± 5.2 mm; at 9 months it was 17.0 ± 5.1 mm; and at 12 months it was 16.7 ± 5.4 mm. There was no significant increase in the mean reaction size to any of the five PPDs applied. However, the difference among subjects was significant at the 0.005 level (ANOVA). Individual patients had relatively small variations in their PPD reaction size. The SD of a random observation within patients was 5.3 mm for the entire study population. Twenty-nine of the 42 patients had differences between extreme values of ±6 mm.

The largest increase in PPD reactivity over time was observed in patients with PPD1 reactions below 15 mm (Fig 2). However, this increase was not statistically significant. Of the two patients who remained negative 2 weeks after PPD2 was applied, one patient became positive on retesting after 3 months of treatment and one patient remained negative throughout the study period and subsequently died due to a relapse of his pulmonary tuberculosis. His tuberculin reactions ranged between 2 and 4 mm. Of 30 patients with hypersergic reactions at entry, 27 remained hypersergic during the entire observation period.

In patients followed up for 1 year, the mean tuberculin reaction size of PPD1 was 16.7 ± 5.4 mm for 26 patients without BCG scars and 14.4 ± 5.0 mm for 16 patients with BCG scar(s). Only the BCG-immunized group had significant differences (p < 0.003) within subjects, due to an increase in reaction size over the 12-month observation period (Fig 3).

DISCUSSION

Our results show that there is a wide distribution of tuberculin reaction sizes at the time of diagnosis in patients with active pulmonary tuberculosis. No significant differences were seen between patients with and without BCG immunization. In healthy individuals studied in the same geographic area as our study, the size of the tuberculin reaction correlates with the number of BCG scars present from vaccination at birth, 6, and 14 years of age. The mycobacterial exposure in pulmonary tuberculosis is obviously sufficient to erase the difference in tuberculin reactivity due to differences in the BCG immunization status.

The age of BCG-immunized patients in our study was significantly lower than the age of unimmunized patients. This age difference is consistent with the fact that the BCG immunization program reached 85 percent of the Chilean population in 1985, increasing steadily since its inception 30 years prior to our study.

Whether tuberculin reactivity induced by BCG alone or in combination with subclinical infection is associated with protective immunity remains controversial and was not addressed in the current study. All 23 patients with BCG scars were reactive to tuberculin at the time of diagnosis. However, in retrospective studies like ours, it is impossible to establish if tuberculin reactivity was present at the time of initial exposure and infection with *Mycobac-*
We found negative tuberculin reactions at the time of diagnosis in only 8.6 percent of our patients, possibly reflecting the exclusion of patients with systemic tuberculosis and/or concomitant debilitating diseases. Furthermore, patients with an initial tuberculin reaction ≤15 mm showed only a small increase in tuberculin reactivity 2 weeks later. Negative tuberculin skin tests among patients with tuberculosis have been reported in several studies. The lack of large increases in tuberculin reactivity within 2 weeks of treatment observed in our patient population differs from the findings of other authors. Rooney et al reported that 25 percent of 100 patients with active tuberculosis were tuberculin-negative when tested within 24 h of hospital admission. After 2 weeks of treatment, the vast majority of these patients had developed positive reactions. Daniel et al reported lower responses in Bolivian patients tested within the first 4 weeks of treatment than in patients tested later in the course of the disease (12.0 and 16.7 mm, respectively). Nash and Douglass found that 49 of 200 patients with active pulmonary tuberculosis failed to respond to intermediate strength PPD (5TU). Thirty of these patients demonstrated positive reactions when retested with 250 TU PPD. Transient anergy to tuberculin testing associated with immunosuppressive mechanisms is seen in a higher proportion of patients with miliary, pleural, and meningeal involvement. Our study suggests that pulmonary tuberculosis alone does not induce mechanisms that suppress tuberculin reactivity.

Our results also suggest that tuberculin testing does not have a boosting effect in patients with pulmonary tuberculosis. We and others have described such an effect of tuberculin testing in healthy, BCG-immunized individuals. We found that the increase in reaction size of a second tuberculin test applied 2 weeks after the first depended both on the BCG immunization status and on the size of the initial tuberculin reaction. The largest increases were seen in individuals with prior BCG immunization who were initially negative (<10 mm). Unimmunized individuals and BCG-immunized individuals with positive (≥10 mm) reactions to the first tuberculin test had only minor increases when retested 2 weeks later. Our results in this study of patients with active pulmonary tuberculosis further support the suggestion that individuals who are already reactive to tuberculin by virtue of mycobacterial infection do not experience a further enhancement of their tuberculin reactivity because of sequential tuberculin testing.

Prolonged follow-up of our patients over 1 year revealed small increases in reactivity after treatment only in patients whose initial reaction was below 15 mm. Hyperergic patients had virtually no change over the entire observation period. Repeated tuberculin testing of elderly persons without tuberculosis increases the number of positive reactions. However, the percentage converting with each test is progressively smaller. Our observation in patients with pulmonary tuberculosis suggests that hyperergic tuberculosis patients have a maximum expression of tuberculin reactivity that does not increase with repeated tuberculin testing.

The finding of persistent tuberculin reactivity in patients with pulmonary tuberculosis stands in contrast to observations of skin test reversion in individuals treated with chemoprophylaxis. When hospital employees were tuberculin tested again after 1 year of receiving isoniazid chemoprophylaxis, 50 percent reverted to negative, 25 percent had decreased reactions, and 25 percent remained unchanged. In another study, four sequential tuberculin tests at 3-month intervals revealed transient negativization of tuberculin reactivity in four of ten hospital employees receiving isoniazid chemoprophylaxis. In our population, only 3 of 38 patients who were initially positive reverted to negative values 1 year after the initial PPD. This observation suggests that tuberculin reactivity in patients with proven pulmonary tuberculosis may last longer than in individuals who become tuberculin reactive without developing disease after exposure to M tuberculosis and who receive chemoprophylaxis.

The observed increase in tuberculin reactivity over 1 year in the BCG-immunized group may reflect the lower tuberculin reactivity at entry in this group. This puzzling observation may indicate that BCG-immunized individuals who have development of tuberculosis may have conditions that decrease tuberculin reactivity and contribute to the failure of BCG immunization to protect against the development of tuberculosis. The low prevalence of HIV infection in Chile at the time of our study suggests that progressive immunodeficiency caused by HIV infection is not the cause for BCG failures in our population. Further studies of BCG failures are required to determine their cause and mechanism.

The relatively small intrapatient variation of tuberculin reactivity observed in patients followed up over 12 months further suggests that each individual has a relatively constant tuberculin reactivity that is expressed under conditions of maximal mycobacterial exposure. However, some individuals may express persistent, intense tuberculin reactivity even in the absence of a detectable, high mycobacterial load. In studies of elderly individuals receiving three sequential tuberculin skin tests, initially positive tests are more likely to remain stable 1 year later than tests that were increased to positive reactions only after the first administration. We made a similar observation...
in healthy first-year university students in Santiago,
Chile. All students who were hyperergic at admission
remained hyperergic 1 year later.1 Taken together, our
results in tuberculosis patients and published results
in healthy BCG-immunized and unimmunized individu-
als suggest that studies of the genetic regulation of
the intensity of tuberculin reactivity to mycobacteria
need to consider the BCG immunization status of a
population, the mycobacterial load at the time of
testing, transient immunoregulatory phenomena, and
also the stability of tuberculin reactivity over pro-
longed periods of time.

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