COINCIDENT FILARIAL, INTESTINAL HELMINTH, AND MYCOBACTERIAL INFECTION: HELMINTHS FAIL TO INFLUENCE TUBERCULIN REACTIVITY, BUT BCG INFLUENCES HOOKWORM PREVALENCE

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Abstract. The prevalence of helminth and tuberculosis infections is high in South India, whereas Bacille-Calmette-Guerin (BCG) vaccine efficacy is low. Our aim was to determine whether concurrent helminth infection alters the ability to mount a delayed-type hypersensitivity response to tuberculin. In a cross-sectional study in southern India, individuals 6–65 years of age were screened for intestinal helminths, circulating filarial antigenemia, tuberculin reactivity, active tuberculosis, and history of BCG vaccination; 54% were purified protein derivative (PPD) positive, 32% had intestinal helminth infection, 9% were circulating filarial antigen positive, and 0.5% had culture-confirmed active tuberculosis. Only age and BCG vaccination were significantly associated with PPD reactivity; however, BCG vaccination was associated with a lower prevalence of hookworm infection relative to those without prior BCG vaccination. Neither intestinal helminth infection nor filarial infection was associated with diminished frequencies of PPD positivity. Our findings suggest that preceding helminth infection does not influence significantly the delayed-type hypersensitivity response to tuberculin.

INTRODUCTION

Infection with tissue-invasive and/or intestinal helminths, important causes of morbidity, affects a large proportion of human populations living in tropical and subtropical regions of the world.1–3 These have been associated with growth stunting,4–6 with poor cognitive performance in children,7–9 and with malabsorption.6,10–12 Infection with Mycobacterium tuberculosis represents one of the most important causes of morbidity and mortality, with 95% of infected persons living in developing regions.13 Based on World Health Organization estimates, 8.8 million new cases of tuberculosis occurred globally in 2003, with the greatest disease burden in Southeast Asia.14 While there are several examples of how immune imbalance created by a parasitic infection can affect subsequent responses to disparate immunologic stimuli,15,16 it should also be noted that the failure in tropical countries of certain vaccines shown to have efficacy in nontropical countries—such as Bacille-Calmette-Guerin (BCG) in South India17 and oral cholera vaccine in South Americans of low socioeconomic status18—has suggested that concurrent helminth infection may play an important role in altering the immune response to both exogenously administered antigens/vaccines and to naturally acquired infections.19,20

Infections caused by intestinal helminths are very common in South India. One study21 estimated the prevalence of hookworm infections to be 62% in an area near Vellore, India, whereas the prevalence of Strongyloides and Ascaris was lower (15.4% and 6.4%, respectively). In an area of similar socioeconomic status near Chennai, India, the prevalence of exposure to mycobacterial antigens, as measured by reactivity to purified protein derivative (PPD), in the general population is estimated to be virtually 100% to PPD-B and about 60–70% to PPD-S by age 24 years.22 Both tuberculosis and helminth infections have been studied extensively, but information is sparse regarding coinfection in coendemic regions.23–25 Based on age prevalence curves, many helminth infections seem to be acquired before M. tuberculosis infection. Moreover, there is growing evidence that the stereotypic immune responses associated with both intestinal and tissue-invasive systemic helminths (e.g., filariae) might influence the delayed-type hypersensitivity (DTH) skin test responses to M. tuberculosis. In addition, the bystander suppression found as a consequence of helminth antigen-specific immune responses13 could alter fundamentally the interferon (IFN)-γ responses necessary to contain M. tuberculosis.26

Given the known failure of live BCG vaccination in South India in an area in which inhabitants harbor intense geohelminth infections and in whom 10–20% have active filarial infection as well, the purpose of this study was to examine the interactions between concurrent intestinal and/or filarial infection on PPD reactivity. More specifically, we have attempted to demonstrate the influence of these helminth infections on modulating the DTH response to tuberculin.

MATERIALS AND METHODS

Study population. In preparation for an intervention trial of antifilarial therapy in PPD-negative helminth-infected patients, a community-based assessment was performed to estimate the prevalence of coinfection with filarial and/or intestinal helminths and latent tuberculosis. The target population comprised all persons 6–65 years of age from five villages of the Tiruvallur District, Tamil Nadu, located 40 km from Chennai, India. The study population was comprised of those eligible persons (7397) who consented to study participation. Recruitment was conducted from June 1999 through April 2000, and a total of 5,096 persons were enrolled in the study. Each study participant received a complete medical evaluation including detailed medical history and physical examination directed toward assessment of lymphatic filariasis (LF) symptoms. Demographic, clinical, and epidemiologic information was recorded onto standardized forms. History of BCG vaccination was assessed by examination of subjects’ arms for the characteristic BCG scar. All study subjects had one stool examination for parasitic ova and larvae. Whole
blood (1 mL) was obtained for determination of circulating filarial antigen (CFA) levels and hematocrit. In addition, all subjects were tested for latent tuberculosis through assessment of skin test reactivity to intradermal PPD. A positive PPD skin test was defined as an induration at the site of inoculation of at least 12 mm diameter to account for the high prevalence of environmental mycobacteria. This cut-off was based on previously determined norms for South India. Any person with pulmonary symptoms (cough, fever, chest pain, hemoptysis) and/or an abnormal chest radiograph had two sputum cultures taken for detection of M. tuberculosis by smear microscopy and culture. Socio-economic status was not measured and therefore was not included in the multivariate analysis because of homogeneity within the study population.

Parasitologic examination. Single stool samples were collected, transported to the laboratory at ambient temperatures, and examined by direct microscopy and by formal-gasoline concentration techniques. Wuchereria bancrofti infection was determined by measurement of CFA using an immunocromatographic test (Binax, Portland, ME) on whole blood.

Tuberculin skin testing, BCG vaccination status, and diagnosis of active tuberculosis. Tuberculin PPD (0.1 mL) of Tween RT23 was injected intradermally on the dorsal aspect of the right arm. After 48 hours, the induration was measured. BCG vaccination status was determined by the presence of a scar.

The presence of active tuberculosis was based on smear microscopy for acid-fast bacillus (AFB) using Ziehl-Neelsen staining followed by fluorescence microscopy and culture using a modified Petroff method for culture of M. tuberculosis.

Data entry and statistical analysis. Data were entered into the Datastar package and verified by double entry. For purposes of analysis, we defined PPD positivity as an induration of 12 mm or greater. Univariate odds ratios (ORs) were calculated. As part of this univariate analysis, these variables were screened for inclusion in a multivariate model, with a value of $P < 0.05$ used as the cut-off point for statistical significance. Age, sex, and BCG vaccination status were included in all multivariate models because they were identified as confounders. A series of logistic regression models were fit by both forward and backward selection. Hookworm and circulating filarial antigen were not significant in multivariate analysis but were forced into the model to obtain point estimates. Likelihood ratio test comparisons were used to find the best model fit for the data. Crude and adjusted OR and 95% confidence intervals (CIs) are reported. Data analysis was performed using SAS (v 8.2).

RESULTS

Demographics and epidemiology of the study population. Study subjects ($N = 5096$) were screened for intestinal parasites, CFA, PPD reactivity, BCG vaccination status, and active tuberculosis (Table 1). Among 5096 subjects, 4456 persons had results for all screened variables and were included in the analysis. Study participants ages ranged from 6-65 years; the mean age of the study population was 29 years. CFA was present in 9% of the entire study group and in 12% with filaria-associated symptoms. As can be seen, the majority of those with disease associated with LF had adenolymphangitis (by history). Hydrocele was the second most common manifestation. Thirty-two percent of the study population had one or more intestinal helminth infections, whereas 31% of the study group ($N = 1,363$) had been vaccinated with BCG at birth (Figure 1). Fifty-four percent were PPD positive, and 0.5% of the subjects had culture-confirmed tuberculosis. The prevalence of co-infection with $M. tuberculosis$ (defined as positive PPD) and hookworm and/or LF was 20% (Table 1). Nearly one third (32%, $N = 1,431$) of the 4,456 study subjects had intestinal helminths, with hookworm accounting for the overwhelming majority (30%) of these geo helminths. Other parasites detected included Trichuris trichiura (0.63%), Hymenolepsis nana (0.61%), Ascaris lumbricoides (0.43%), Enterobius vermicularis (0.23%), Strongyloides stercoralis (0.06%), and Taenia spp. (0.04%). Consequently, further analyses of stool parasites were restricted to the presence of only hookworm in stool, although inclusion of other intestinal parasites did not change the findings (data not shown).

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<td>Characteristics of study population ($n = 4,456$)</td>
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FIGURE 1. Age-specific prevalence of BCG vaccination in men (open bars) and women (closed bars).
The prevalence of hookworm infection for women was 1.1 times that for men (Figure 2A). The overall prevalence of age- and sex-specific hookworm infection ranged from 26% to 40% in women and from 25% to 32% in men. Filarial infection prevalence among persons 6–19 years of age was 7.2%. After age 19, the prevalence of filarial infection increased among men and was 2-fold that compared with women 20 and 49 years of age (Figure 2B). A total of 402 of 4,456 (9%) had CFA. Although most of these CFA-positive subjects were clinically asymptomatic, 363 of 4,456 study subjects (8%) had clinical manifestations associated with LF (e.g., hydrocele, lymphedema, elephantiasis). Only a small percentage (12%) of those with clinical signs or symptoms associated with LF had evidence of active infection based on CFA. PPD reactivity increased with age and doubled between the second and third decade of life. By age 30 years, 60% of subjects were PPD positive (Figure 2C). BCG vaccination status varied greatly among age groups. Overall, 40% of persons more than 30 years of age had evidence of having been vaccinated with BCG. The cohorts 15 to 29 years of age generally had quite low vaccination rates, 10–20%, whereas in the youngest age group studied, the 6 to 9 year olds, vaccination at birth with BCG was nearly 40%. The prevalence of intestinal helminth infection peaked earlier than that of PPD reactivity.

BCG vaccination was strongly associated with PPD reactivity in univariate analyses (OR = 1.6; 95% CI, 1.4, 1.8). Most important, BCG vaccination at birth was significantly protective for hookworm infection (OR = 0.80; 95% CI, 0.69, 0.92) across all age groups. Those never vaccinated with BCG but PPD positive were 1.3 times more likely to be infected with intestinal helminths (OR = 1.3; 95% CI, 1.0, 1.6). PPD-positive persons were also 20% (OR = 1.2; 95% CI, 1.0, 1.5) more likely to have CFA in their blood. The presence of hookworm infection and/or filarial infection did not seem to influence the presence of active tuberculosis (data not shown).

**Multivariate analyses.** Based on the results from univariate analyses to identify potential confounders, a multivariate logistic regression model was created adjusting for gender, age, BCG vaccination, and helminth infection. In addition, the effect of age/gender interactions was accounted for by including an age/gender interaction term. PPD positivity was significantly associated with BCG vaccination status and age. Moreover, a trend of increasing PPD reactivity with increasing age was observed for both men and women, although this association was much stronger among men over age 30 compared with women over age 30. Hookworm infection, filarial infection, and gender were not significant predictors of PPD positivity (Table 2).

After controlling for PPD positivity, CFA, age, gender, and age and gender interactions, BCG remained significantly protective for hookworm acquisition (Table 3). After controlling for age and gender interactions, gender was not significantly associated with hookworm infection. Increasing age did not seem to be a significant predictor of hookworm acquisition among men, but there was a significant association between hookworm acquisition and age among women over 30 years. Additionally, CFA was significantly associated with hookworm acquisition (Table 3).

**DISCUSSION**

We found that BCG vaccination has a protective effect against hookworm infection. Hookworm represented the overwhelming burden of intestinal nematode infection, with other nematodes representing only a small fraction. Additionally, we showed that having been BCG vaccinated was an-
other important predictor for PPD positivity in this South Indian population. While neither sex, intestinal helminth, nor filarial infection was a significant predictor of skin test positivity to PPD, BCG vaccination and age were significantly associated with PPD positivity. While the socio-economic level was relatively homogeneous in this population with regard to living conditions and educational level, slight variations in socio-economic could affect findings on helminth acquisition. However, socio-economic data were not collected and are thus a potential limitation of our study.

The protective association between BCG vaccination and reduced odds of intestinal helminth infection found in this population has been identified in other parts of the world as well. Barreto et al. showed that Brazilian children who had been BCG vaccinated at birth (using the presence of a BCG scar as an indicator) had a significantly lower hookworm incidence, prevalence, and intensity of infection than those who had not been vaccinated. A Ugandan HIV/AIDS study found that those previously vaccinated with BCG also had a lower prevalence of nematode infection than those who were unvaccinated; however, this protective effect has not been consistently identified across regions and study areas, with studies in Malawi and Ecuador finding no effect of BCG on intestinal helminth infection. Some of the differences among the various studies might be explained by differences in helminth prevalences or other host/helminth interactions. Although it is not clear why BCG vaccination provides protection against hookworm acquisition, understanding the relationship between early childhood exposure to bacterial pathogens and/or endotoxin and atopy may provide insight into the mechanism of this protective effect.

Recent cohort data from many industrialized countries have shown steady increases in atopy and asthma and have led to the hypothesis that a reduction in childhood exposure to infection may prompt the development of atopy. The question of whether BCG vaccination at birth may bias the immune response toward a lasting type 1 response and consequently decrease susceptibility to helminth infection is still unanswered. In large studies in both Denmark and Greenland, no association with BCG vaccination in infancy and development or reduction of allergy or asthma has been shown, whereas a weak protective effect of BCG vaccination against asthma has been found in a study from Germany.

Both atopy and helminth infections are associated with similar immune response profiles (so-called Th2 responses), although the magnitude of these responses is much greater for helminth infections than for allergic diseases. Mycobacterial infections and BCG vaccination, in contrast, primarily induce type 1 (IFN-γ-dominated) CD4+ responses. The interaction between these two opposing arms of the CD4+ response and the presence of other T cells (Treg, Th3, Tr1) capable of modulating both type 1 and type 2 responses suggest that the balance among these various T cells and their cytokine products dramatically influences the ability to handle pathogens of diverse genera. The demonstration of a protective effect of tuberculin reactions on atopy in Japan suggested that the decline of tuberculosis may be responsible for a rise in atopic disorders as atopy could be limited by Th1-dominated immune responses.

In this study, hookworm was the predominant intestinal helminth infection seen, concordant with some studies performed in the past from quite similar regions. A rural South Indian study found hookworm to be the most common intestinal helminth infection (61.5%), with a higher prevalence seen among older age groups. A study in Central India found hookworm and H. nana to be the most common helminths found among adolescent boys and girls. It was surprising, however, that Ascaris and Trichuris represented such a small percentage of the infections seen in our study population, given that in other parts of India, Ascaris and Trichuris are quite common.

Our data on filarial infections were quite consistent with studies done previously in this region of South India. Based on findings from published community-based studies, bancroftian filariasis has a 5% overall prevalence rate in India. In the region of South India studied here, prevalences (based on less-sensitive microfilaria-carriage rates) have ranged from 4% to 20% (V. Kumaraswami, unpublished data). Thus, our 9% rate for CFA is quite consistent with these previous findings. Similar to other studies, this study also found the prevalence of filarial infection to be somewhat greater in men than in women, particularly during the reproductive years. The prevalence of symptomatic infection for LF was higher in men, primarily because of the inclusion of hydroceles as one clinical manifestation of LF.
Because PPD reactivity is a screening tool for latent tuberculosis, it is important to control for and address the association between PPD reactivity and BCG vaccination when analyzing the protective effect of BCG vaccination on intestinal helminth acquisition. It is important to note, however, that not all of BCG vaccinees retain a permanent scar and thus slight misclassification of BCG-vaccinated compared with unvaccinated persons might have occurred. While some studies have found a positive association between BCG and PPD reactivity, others claim that BCG vaccination should not be considered in the interpretation of a positive PPD test.52 There is evidence that tuberculin reactivity in persons who have been BCG vaccinated after birth wanes rapidly,53 although other studies suggest that it persists.54 In a meta-analysis examining the effect of BCG on tuberculosis skin test measurement, vaccination with BCG significantly increased the likelihood of a positive tuberculin test55 among persons without active tuberculosis. In our data, while the trend of PPD reactivity increases with age in a linear fashion, the distribution of BCG vaccination did not. It should be mentioned that BCG coverage in the study population is relatively low (31%). The age-specific BCG vaccination coverage in our study likely reflects a reaction to the 1960s BCG trial in which BCG was found to be ineffective against adult pulmonary tuberculosis.17 As a result, routine BCG vaccination was applied less universally and coverage in subsequent birth cohorts declined sharply. This likely explains the low vaccination coverage among persons 15–29 years of age, born between 1971 and 1985, in this study.

The differences in interpreting tuberculin skin test reactivity of BCG-vaccinated versus unvaccinated populations remains controversial; despite these difficulties, however, PPD remains the primary screening tool for tuberculosis detection in both developing and developed regions. Numerous studies have been performed to estimate tuberculosis prevalence based on positive Mantoux skin test results. Tuberculin reactivity appears to be associated with age, and Neuenschwander et al.36 found that in South Korea, M. tuberculosis prevalence increases with age, with prevalence ranging from 5.2% to 7.5% in subjects 0–4 years of age and approaching 90% in the oldest age group, 25–29 years of age. Consistent with these findings, we found a strong linear increase of PPD positivity with age, with 10% prevalence by age 6 years.

The relationship between intestinal helminths and BCG is important, because the greatest need for an effective vaccine for M. tuberculosis exists within those regions with the greatest helminth burdens. The high prevalence of helminths and M. tuberculosis in this rural region of South India represents a high infection burden in this population. Preexisting helminth infection may alter the immune response to PPD but it may not protect against M. tuberculosis exposure or infection. Our results nonetheless indicate that BCG vaccination status and PPD positivity were significantly associated and that BCG seemed protective against hookworm acquisition. The mechanisms behind these interactions are currently under study. Thus, it will be important to pursue more focused immunologic studies to clarify the biologic underpinnings of these relationships as well as further epidemiologic studies to focus on the significance of these multiple interactions.

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Informed consent was obtained from all study subjects before enrollment; for those under the age of 18, parental consent and assent from minors was obtained.

The study protocol was reviewed and approved by the Institutional Review Boards of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, and by the Tuberculosis Research Centre Chennai India; the protocol was also approved by the Health Ministry Screening Committee, New Delhi, India.

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