CONCISE COMMUNICATION

Lower Hookworm Incidence, Prevalence, and Intensity of Infection in Children with a Bacillus Calmette-Guérin Vaccination Scar

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Bacillus Calmette-Guérin (BCG), the most common vaccine worldwide, has broad effects on the immune system. Hookworm infections are a major source of morbidity. In response to a preliminary report of BCG vaccination protection against nematodes in human immunodeficiency virus–infected adults, data from an ongoing prospective study were analyzed to determine the intensity (eggs per gram of stool), prevalence, and incidence of different helminths in children with and without a BCG vaccination scar. Adjusted prevalence and incidence ratios were estimated by using logistic regression. Children with a BCG vaccination scar were found to have statistically significantly lower hookworm prevalence (41%), incidence (37%), and mean egg counts (39%), after controlling for age, sex, and socioeconomic factors. There was no BCG association with incidence, prevalence, or intensity of infection with *Schistosoma mansoni, Ascaris lumbricoides,* or *Trichuris trichiura.* Such protection would have implications for public health and for research on mechanisms behind human immunological responses to hookworm.

Bacillus Calmette-Guérin (BCG) is the most widely used vaccine worldwide. Originally only a vaccine against tuberculosis, BCG has been shown to protect against leprosy [1] and Buruli ulcer [2], to reduce atopy [3], and to improve survival in bladder cancer [4]. A preliminary report of a protective effect of BCG vaccination on the prevalence of intestinal nematodes (*Strongyloides stercoralis* and hookworm species) in human immunodeficiency virus (HIV)–infected persons in Uganda has recently been published [5].

In this paper, we evaluate the effect of BCG vaccination on the incidence, prevalence, and egg burden of hookworm (in Brazil, predominantly *Necator americanus*), *Ascaris lumbricoides, Trichuris trichiura,* and *Schistosoma mansoni,* using a cross-sectional study design with a 1-year follow-up.

Methods

This analysis was undertaken in the population of an ongoing randomized control trial of antihelminthic treatment on nutritional status, which was being conducted in the Brazilian town of Jequié. The study population ($n = 1766$) was a subsample of a survey of 13,771 children, 7–18 years of age, for whom a stool sample was examined. The study population was selected to include children with at least 1 geohelminth (i.e., hookworm, *A. lumbricoides, T. trichiura;* $n = 6269$). Of the 6269 children with geohelminths, 1766 were selected, using numbers generated randomly and the children's preassigned identification numbers. Children who had no intestinal nematode eggs in stools (independent of the presence of *S. mansoni*), who had a high intensity of infection (eggs per gram of stool) or a history of neurological disturbance, or who were pregnant were not included. Complete data on the relevant variables were available, and a 1-year follow-up was performed, for 1529 (86.6%) of the original 1766 children. The population was distributed over 6 neighborhoods.

Two stool samples were collected at recruitment and at 6 and 12 months. For each stool sample, 2 slides were made, which were examined by using the quantitative Kato-Katz method [6]. The slides were read within the first 2 h of preparation, for hookworm egg identification, and the number of eggs for each helminth in

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This project was approved by the Human Investigation Boards of the Centro de Pesquisa Gonçalo Muniz–Fundação Oswaldo Cruz, Brazil, and Case Western Reserve University, in compliance with the US Department of Health and Human Services guidelines. Written informed consent was obtained from the guardians of all subjects.

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Table 1. Prevalence ratio (PR) and risk ratio (RR) of infection with hookworm, *Ascaris lumbricoides, Trichuris trichiura,* and *Schistosoma mansoni* in children, relative to bacillus Calmette-Guérin (BCG) vaccination scar status before and after adjusting for confounding variables.

	Prevalence, %			Annual risk, %		
Parasite	BCG scar	No BCG scar	PR (95% CI)	BCG scar	No BCG scar	RR (95% CI)
Hookworm	13.8	24.7	0.55 (0.42–0.73)[a]	15.7	27.3	0.57 (0.42–0.78)
			0.56 (0.42–0.75)[b]			0.60 (0.43–0.82)
			0.58 (0.41–0.81)[c]			0.61 (0.42–0.90)
			0.59 (0.42–0.83)[d]			0.63 (0.42–0.93)
			0.57 (0.41–0.82)[e]			0.61 (0.42–0.90)
S. mansoni	54.8	62.1	0.88 (0.78–0.99)[a]	29.8	21.9	1.36 (0.84–2.20)
			0.97 (0.94–1.00)[b]			1.34 (0.89–1.99)
			0.99 (0.93–1.05)[c]			1.54 (0.92–2.56)
			0.99 (0.97–1.02)[d]			1.45 (0.92–2.26)
T. trichiura	74.4	74.2	1.00 (0.91–1.09)	28.2	35.6	0.79 (0.51–1.22)
			0.99 (0.93–1.07)			0.88 (0.61–1.28)
			1.00 (0.92–1.08)			0.84 (0.51–1.39)
			0.99 (0.93–1.06)			0.86 (0.55–1.33)
A. lumbricoides	64.5	60.6	1.06 (0.99–1.19)	34.4	33.3	1.03 (0.71–1.47)
			1.07 (0.97–1.19)			0.98 (0.73–1.31)
			1.06 (0.92–1.22)			0.95 (0.65–1.39)
			1.05 (0.96–1.15)			0.96 (0.71–1.28)

NOTE. CI, confidence interval.

[a] Crude value.

[b] Adjusted for neighborhood of residence.

[c] Adjusted for age, sex, family income, education of the head of the household, and sewage disposal method.

[d] Adjusted for age, sex, family income, education of the head of the household, sewage disposal method, and neighborhood of residence.

[e] Adjusted for age, sex, family income, education of the head of the household, sewage disposal method, and *S. mansoni* infection status.

each slide was recorded. Every 10th slide was reexamined, for quality control. The Kato-Katz method is not suitable for identification of larval forms, which is necessary for diagnosis of *S. stercoralis* infection. Thus, *S. stercoralis* was not evaluated. Hemoglobin level was measured in fingerprick blood for all children, using a HemoCue hemoglobinometer system (HemoCue, Angelkolm, Sweden). In a randomly selected subsample of 250 study children, a second blood sample was collected, and the serum was separated, frozen, and later tested for iron, total iron-binding capacity, transferrin saturation, and albumin. Anthropometric measurements were made at entry for all children. During a home visit at baseline, interviewers noted on a standard form specific characteristics of the residence, such as the material used in the house construction, and collected information on family income and other socioeconomic variables. At the 12-month follow-up contact, trained nurses inspected study subjects for a BCG vaccination scar, without knowledge of stool results.

The data were double-entered and cross-checked. The prevalence of infection was calculated as the proportion of children in the population who were positive for the helminth on at least 1 of the 4 stool slides collected at entry. Incidence of infection was calculated as the annual risk of infection—that is, the number of children negative at recruitment who subsequently had at least 1 stool slide that was positive for a helminth at the 6- or 12-month follow-up, divided by the number of children negative for that helminth at entry. Intensity of infection was expressed in eggs per gram of stool and was calculated as the average number of eggs in the 4 slides collected at entry, multiplied by 24 [6].

The association between a BCG vaccination scar and prevalence and incidence of infection with each helminth species was calculated as a crude measure and was adjusted for age, sex, education of head of household, family income, sewage disposal method, and neighborhood of residence, using unconditional logistic regression. These 6 variables were kept in the logistic regressions because they were considered to be potential confounding variables. Three adjusted estimates were made, controlling for different combinations of potential confounders to show the degree of variation in the estimates. To explore whether the sampling method introduced selection bias, hookworm prevalence and incidence ratios were estimated separately in children with and without *S. mansoni* infection. The association was expressed as the prevalence ratio (PR) and the risk ratio (RR). BCG vaccination protection was calculated as 1-PR and 1-RR. The association between BCG scar and intensity of infection was examined by comparing geometric means and using the Student's t test.

Results

The prevalence of BCG vaccination scars decreased with age ($P < .001$), and it was slightly higher in children living in households with inadequate sewage disposal ($P = .013$). There was no significant variation in prevalence of scar according to sex, family income, or education of the head of the household, or with anthropometric or biochemical indicators (data not shown).

There was a significant and marked reduction in prevalence

Table 2. Geometric mean of eggs per gram of stool in children infected by hookworm, *Ascaris lumbricoides, Trichuris trichiura,* or *Schistosoma mansoni,* according to bacillus Calmette-Guérin (BCG) vaccination scar status.

	BCG scar			No BCG scar			
Parasite	No. of children	Eggs/g	95% CI	No. of children	Eggs/g	95% CI	*P*
Hookworm	191	150.3	122.2–184.9	49	247.6	160.8–381.4	.036
S. mansoni	759	65.8	61.8–70.2	123	71.6	61.4–83.6	.332
A. lumbricoides	893	2387.8	2122.4–2686.4	120	2236.1	1592.4–3140.2	.881
T. trichiurus	1030	223.7	205.1–243.9	147	227.8	184.4–281.5	.709

NOTE. CI, confidence interval.

of hookworm (44% protection) in children with a BCG vaccination scar. The protection remained statistically significant after controlling for age, sex, family income, education of the head of the household, sewage disposal method, and neighborhood of residence and after the inclusion of *S. mansoni* infection in the model. Initially there was a statistically significant reduction in the prevalence of *S. mansoni* in children with a BCG vaccination scar, but this disappeared after adjustment for confounders. No effect of BCG was observed on either the crude or the adjusted PRs of *T. trichiura* and *A. lumbricoides* infection (table 1).

The effect on incidence of infection with hookworm was statistically significant, similar to that on prevalence (43% protection), and it remained statistically significant after controlling for the 6 potential confounding variables (37% protection) and after inclusion of *S. mansoni* infection status in the model. There was a nonsignificant increase in incidence of *S. mansoni* in children with a BCG vaccination scar, no effect on *A. lumbricoides,* and a small, nonsignificant protective effect against *T. trichiura.* These were not changed by control of potential confounding factors (table 1).

A separate analysis was performed to estimate the effect of BCG vaccination on hookworm in children with and without *S. mansoni* infection, adjusting for the 6 variables. In infected children, the PR was 0.65 (95% confidence interval [CI], 0.44–0.97), and the RR was 0.58 (95% CI, 0.37–0.91). In uninfected children, the PR was 0.47 (95% CI, 0.25–0.85), and the RR was 0.68 (95% CI, 0.34–1.36).

The geometric mean of hookworm egg counts was 37% lower in infected children with a BCG vaccination scar than in those without a scar ($P = .036$). The geometrical means of egg counts for *S. mansoni, A. lumbricoides,* and *T. trichiura* were not significantly different between infected children with and without a scar (table 2).

Discussion

The study found a robust and statistically significant reduction in prevalence, incidence, and intensity of infection with hookworm in children with a BCG vaccination scar. This is consistent with preliminary findings of BCG protection against intestinal nematodes (*S. stercoralis* and hookworm) in HIV-1–infected adults in Uganda [5].

We used BCG vaccination scars as an indicator of BCG vaccination. Although it is possible that development of a BCG scar after vaccination is modified by the same host immune responses that regulate worm infection, lack of response to properly administered and stored BCG vaccine is very uncommon (e.g., 3% in India [7]), and its use is standard practice in retrospective studies of BCG vaccination protection [8].

Could the association between BCG vaccination and hookworm be due to bias in the selection of the study subjects? The sample overrepresented children infected by *S. mansoni* and at least 1 nematode and excluded children with no intestinal nematode eggs in stool. BCG effects were similar regardless of *S. mansoni* infection status, and thus it is unlikely that oversampling of *S. mansoni*–infected children introduced selection bias. The absence of a BCG effect against *A. lumbricoides* and *T. trichiura* suggests that the exclusion of nematode-free children likewise did not introduce selection bias. The finding in this study is also consistent with the protection found in Uganda, where uninfected children were not excluded [5].

Could the association between BCG vaccination and hookworm be due to confounding factors? We controlled for age, sex, 3 socioeconomic indicators, and neighborhood of residence. This removed the statistically significant association found with *S. mansoni* prevalence in the univariate analysis but did not change the effect of BCG vaccination on hookworm or the lack of effect found with *A. lumbricoides* and *T. trichiura.* Control for neighborhood of residence should remove unknown confounders that cluster at the neighborhood level, such as distance to health services. We did not have information on risk factors specific to each of the helminths, such as use of shoes for hookworm and water contact for *S. mansoni.* These, however, would confound the association between BCG vaccination and helminth infection only if they were independently associated with frequency of BCG vaccination and did not cluster in neighborhoods. This seems unlikely, and we believe the associations found can be real, particularly in consideration of how remarkably stable the protective effect of BCG was on hookworm after confounding factors were controlled for.

It is not clear why BCG protection was found against hookworm in this study but not against the other helminths. Immunological defense mechanisms against helminths are known to vary from parasite to parasite and in the different developmental stages of the infection [9, 10]. Superficially, the pro-

tection found may appear to be paradoxical. BCG is a strong stimulus for Th1-like responses, in which interferon-γ predominates [11], and it is often stated that Th2 responses play a central role in protection against intestinal nematodes [12]. However, much remains to be clarified about the relevant effector mechanisms behind protective immunity against intestinal nematodes, and the little that is known comes from experiments in vitro and in gene-deficient mice [13]. Even in these experimental situations, a complex interplay between Th1 and Th2 pathways has been found [14].

This study provides strong epidemiological evidence of a lower prevalence, incidence, and intensity of infection in children with a BCG vaccination scar, consistent with a protective effect of BCG against hookworm infection. It is important that other epidemiological studies be undertaken to further explore this finding. BCG protection against hookworm would have public health implications, strengthening the case for continued BCG vaccination in developing countries, and it could contribute to our search for understanding of the mechanisms behind BCG protection and behind human immunological responses to hookworm.

Rapid development of immunological techniques and theories has created many opportunities for fruitful collaboration between immunology and epidemiology. One of the roles of epidemiology in this collaboration is to identify associations and patterns that can generate and focus immunological research, in the same way that immunological theory and results should influence epidemiological research by generating new hypotheses and informing the interpretation of results. The epidemiological finding reported here—particularly if it is confirmed elsewhere—poses a challenge to immunologists and molecular biologists to clarify the biological mechanisms underlying this protective effect.

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