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Does Previous BCG Vaccination Interfere with the Serodiagnosis of Tuberculosis Using *Mycobacterium tuberculosis*-specific Glycolipid Antigens?

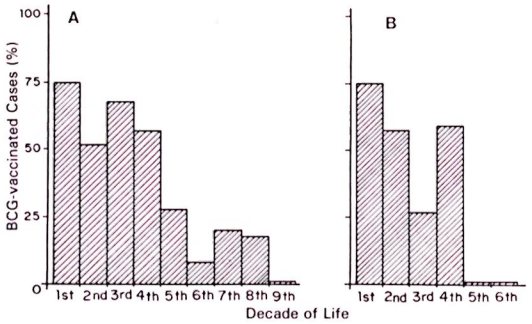
TO THE EDITOR:

There is evidence to the effect that the development of protective T-cell-mediated protective immunity results in suppression of humoral B-cell immunity in leprosy and tuberculosis ([4]). BCG vaccination is believed to induce protective immunity against tuberculosis. It may be reasonably assumed that BCG-vaccinated individuals who subsequently are diagnosed with tuberculosis should build up protective immunity more efficiently than nonBCG-vaccinated individuals. Therefore the sensitivity of serodiagnosis should be lower in BCG-vaccinated tuberculosis patients. To examine this question, we decided to conduct a study in patients residing where BCG coverage was wide and where the incidence of tuberculosis was high as a means of detecting sufficient numbers of tuberculous patients who had received BCG vaccination.

The city of Manaus (Amazonas, Brazil) was selected for the study because, according to the health authorities, about 50% of the population had been vaccinated 1 month after birth and the incidence of smear-positive cases of tuberculosis was estimated at 69.2/100,000 inhabitants for the last decade (ranging from 70.5 in 1981 to 58.9 in 1989). During the same period, the population in the state of Amazonas increased by about 30% (1.5 million in 1981 and 2.0 million in 1989).

As part of the clinical history of all patients who are personally observed at the National Research Institute of the Amazonia (INPA), previous BCG vaccination is registered. Evidence of BCG vaccination is established by searching for the characteristic vaccinal scar in the deltoid region. This scar is easily differentiated from the smallpox vaccination scar which can still be found in the older age groups. The frequency distribution of 273 confirmed tuberculosis cases in respect to previous BCG vaccination and decade of life is shown in The Figure. The proportion of patients who received BCG vaccination was 48.3% and, as shown in The Figure, the proportion of vaccinated patients was over 50% during the first four decades of life, dropping sharply thereafter. Judging from these unexpected findings, we decided that a sample of about 50 successive cases of tuberculosis should be enough to answer the question of whether previous BCG vaccination might interfere with the sensitivity of serodiagnosis. As shown in The Figure, the sampled population was representative of the whole population of tuberculosis patients.

Tuberculosis disease was confirmed in all cases indicated above by isolation and identification of *Mycobacterium tuberculosis* using current methods ([3]). To start the study, blood was collected from successive patients before treatment, and the sera were kept frozen until ready to use. When the total number of bacteriologically confirmed tuberculosis cases reached 53 their sera were used for ELISA. The serology procedure used was as described previously ([1]). The antigens used were the PGL-Tb1 and the SL-IV glycolipids isolated from the strain Cannetti of *M. tuberculosis* ([2, 7, 8]). ELISA



THE FIGURE. Tuberculosis disease in BCG-vaccinated patients. **A** = Frequency distribution of BCG-vaccinated tuberculosis patients with respect to decade of life (number vaccinated, 132; number examined, 273). **B** = Frequency distribution of BCG-vaccinated cases among 53 successive tuberculosis cases selected for ELISA with respect to decade of life (number vaccinated 26).

values were assayed at a 1/250 serum dilution, and were scored as positive if >200 for the PGL-Tb1 and/or > 300 for the SL-IV antigens ([1]).

The ELISA results in the sera of the 53 cases selected for the study are shown in The Table. The sensitivities of serodiagnosis were 26.9% and 37.0% in, respectively, the nonvaccinated and the BCG-vaccinated patients. The difference between the two groups was not statistically significant ($\chi^2 = 3.36$, $\alpha = > 0.05$). We have therefore concluded that previous BCG vaccination did not adversely affect serodiagnosis. However, the conclusion must be tempered because the unexpected high proportion of tuberculosis patients who had been BCG vaccinated suggests that the vaccination was ineffective.

Although the purpose of this study was not to evaluate the efficacy of BCG vaccination, our observations led to the following considerations. It was suggested that BCG vaccination may enhance or depress the protective immunity against tuberculosis that is supposedly induced by environmental mycobacteria ([11]). The occurrence of environmental mycobacteria was also advanced as one possible explanation for the failure of BCG vaccination in the Chingleput, India, evaluation of its efficacy ([6]). Previous studies in Manaus showed that environmental mycobacteria, including potentially pathogenic species, were isolated from 25.4% of all clinical specimens examined ([10]), from 31.3% of hand and forearm washings of healthy individuals ([9]), and from 17.9% of hand washings of leprosy patients ([5]). In view of the above results and considerations, we concluded that an in-depth investigation of this matter is urgently needed in regions like the Amazonia where environmental mycobacteria are highly prevalent. Because of these observations, we began to collect information on previous BCG vaccination in leprosy patients for use in verifying if it might somehow interfere with anti-PGL-I titers in this disease.

THE TABLE. *ELIA results in sera from 53 bacteriologically confirmed cases of tuberculosis according to previous BCG vaccination.*

ELISA	BCG status		Total
	Vaccinated	Non-vaccinated	
Positive	10 (37%)	7 (27%)	17 (32%)
Negative	17 (63%)	19 (63%)	36 (68%)
Total	27 (100%)	26 (100%)	53 (100%)

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Subcorneal Pustular Dermatosis in Type 2 Lepra Reaction

To the Editor:

A 46-year-old male was diagnosed to have lepromatous leprosy in July 1990, based on clinical and histopathological features and by demonstration of acid-fast bacilli in slit-skin and earlobe smears. There were multiple, shiny, infiltrated macules, plaques and nodules distributed bilaterally and symmetrically on the trunk, limbs and face. The bacterial index (BI) was 6+ (Ridley-Jopling scale) and the morphological index (MI) was 40%. Routine laboratory tests on blood, urine and stools were normal. He was treated with dapsone, rifampin and clofazimine as recommended by the World Health Organization (WHO) for multibacillary leprosy. While on treatment he developed features of type 2 lepra reaction characterized by intermittent high fever, anorexia, neuralgia and multiple erythema nodosum leprosum (ENL) lesions on the front of his legs, face and forearms. Along with these, he also developed numerous pin-head to pea-sized, superficial, oval flaccid pustules on his limbs, buttocks and chest. On the buttocks they were arranged in a serpiginous pattern, while on the arms they remained discrete (Figs. 1 and 2). These pustules were independent of the ENL lesions. Each pustule dried up and desquamated in 5 to 7 days leaving faint hyperpigmentation, but fresh crops of pustules continued to erupt along with the features of systemic toxicity. Blood tests done at this time revealed leukocytosis (12,000 cells/cubic mm) with a predominance of neutrophils (70%) in the differential leukocyte count. ESR was 80 mm first hour (Westergren). Blood VDRL and tests for rheumatoid factor and lupus erythematosus (LE) cells were negative. Gram staining of the pus taken from the pustules did not show any bacterium, and its culture was sterile. Histopathological study of an intact pustule showed a subcorneal pustule containing numerous neutrophils (Fig. 3) and diffuse macrophage granuloma in the dermis with a clear subepidermal zone. There were no histopathological features of vasculitis.

The dose of clofazimine was increased to 300 mg daily, and he was also given ibu-