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Prophylactic Effect of *Mycobacterium bovis* BCG Vaccination against Osteomyelitis in Children with *Mycobacterium ulcerans* Disease (Buruli Ulcer)

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***Mycobacterium ulcerans* disease, or Buruli ulcer (BU), causes significant morbidity in West Africa. In 233 consecutive, laboratory-confirmed samples from BU patients in Benin whose *Mycobacterium bovis* BCG scar status was known, 130 children (<15 years old) and 75 adults had a neonatal BCG vaccination scar. Of 130 children with BCG scars, 10 (7.7%) had osteomyelitis, while 3 of 9 children without BCG scars (33.3%) had osteomyelitis. Our observations support the conclusion that having a BCG vaccination scar provides significant protection against *M. ulcerans* osteomyelitis in children with BU disease.**

Mycobacterium ulcerans disease, also known as Buruli ulcer (BU), is the third most frequent mycobacterial disease of humans after tuberculosis and leprosy (17). The etiologic agent elaborates a necrotizing toxin (mycolactone) that destroys skin, subcutaneous tissue, and bone (1, 5). Recently, BU has emerged as an increasingly important cause of morbidity, particularly in West Africa where its increased prevalence is most likely related to a complex of environmental changes (6, 10). In some areas where BU is endemic, the incidence of the disease exceeds those of leprosy and tuberculosis (4). BU affects children under 15 years of age most frequently. Contrary to the name "Buruli ulcer," clinical findings and microbiological and histopathological analyses of cases confirm that 50% of BU cases in Benin present with only nonulcerative lesions (F. Portaels J. Aguiar, M. Debacker, C. Stenou, C. Zinsou, A. Guédénon, and W. M. Meyers, unpublished data). Severe debilitating disease is common; for example, up to 14% of all patients with BU in Benin have osteomyelitis (4).

The epidemiology of BU is only partially understood. There is evidence that the foci of the disease are usually associated with stagnant water in rural settings and that transmission to humans may involve water-dwelling insects (11). The only widely accepted treatment is surgical excision of the lesion and subsequent skin grafting. Excision of lesions frequently leads to deforming scars. Patients with osteomyelitis often undergo amputations.

Control of BU, as with most public health problems, involves multiple, often interrelated socioeconomic, environmental, and biomedical issues. Because BU is not naturally contagious, early identification and treatment of cases alone will not control the disease. Elimination or containment of the etiologic agent depends on favorable environmental alterations, and reduced transmission of the disease will require amelioration of socioeconomic conditions. In areas where BU is endemic, neither of these factors is likely to improve in the near future, making prevention by immunoprophylaxis the only logical approach to disease control.

The pathogenesis of BU lesions is closely related to the action of the necrotizing toxin elaborated by *M. ulcerans* (1). This toxin causes necrosis of skin, subcutaneous tissue, and bone and is immunosuppressive (5, 7, 9). Immunoprophylaxis rationales may be directed to the neutralization of the toxin by humoral antibody or to the induction of a cell-mediated immune capability that would destroy the etiologic agent early in the infection. Vaccines directed toward the neutralization of the toxin have not been studied. However, the following observations suggest that the cell-mediated immune system rationale may be a valid approach to immunoprophylaxis. (i) Lesions of BU heal naturally by development of delayed-type hypersensitivity granulomas (5). (ii) The burulin skin test, which assesses delayed-type hypersensitivity response to several mycobacterial species but putatively is most specific for *M. ulcerans* infections, tends to become positive during BU disease. Stanford et al. (13) found that of 45 patients who were burulin negative before BU disease, 92% of these same subjects had increased burulin reactions after onset of BU disease. (iii) There is T-cell anergy to *M. ulcerans* in BU patients; however, individuals sensitized by *Mycobacterium bovis* BCG show significant T-cell responses in vitro to live *M. ulcerans* (2). (iv) A DNA vaccine encoding antigen 85A of BCG protects against BU infection in mice (14). (v) Two prospective trials show that BCG vaccination confers protection against BU ranging from 18 to 74%, with an overall protection rate of 47%. In addition, vaccinated individuals who developed BU usually developed less severe forms of the disease, and histopathologically, their lesions showed more reactive cellular responses (12, 15).

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TABLE 1. Relationship between osteomyelitis and neonatal BCG vaccination scar in the total patient population

BCG scar	No. of patients (%)		Total no. of patients
	With osteomyelitis	Without osteomyelitis	
Present	25 (12.2)[a]	180 (87.8)	205
Absent	10 (35.7)[a]	18 (64.3)	28
Total	35	198	233

[a] The percentages of patients with osteomyelitis with and without BCG scars were statistically significant ($P = 0.002$) by Fisher's exact test.

TABLE 3. Relationship between osteomyelitis and neonatal BCG vaccination scar in patients at least 15 years old

BCG scar	No. of patients (%)		Total no. of patients
	With osteomyelitis	Without osteomyelitis	
Present	15 (20)[a]	60 (80)	75
Absent	7 (36.8)[a]	12 (63.2)	19
Total	22	72	94

[a] The percentages of patients with osteomyelitis with and without BCG scars were statistically significant ($P = 0.137$) by Fisher's exact test.

Given the protective effect of BCG against disseminated tuberculosis in children, we were prompted to evaluate the possibility of a protective effect of neonatal BCG vaccination against a severe form of BU, osteomyelitis, in children in Benin. BCG vaccination status was assessed by the presence or absence of a typical BCG scar. Reported observations (8, 16) confirm that this is a good indicator of BCG vaccination.

We studied 233 consecutive patients confirmed as BU cases by microbiological (direct smear examination for acid-fast bacilli, culture for *M. ulcerans*, and IS*2404* PCR) and histopathological analyses (18). Only patients who had positive findings for at least two of the four tests were accepted for the study. They were then examined for the presence of a BCG scar. A BCG scar was found in 205 patients (88.0%), and 28 patients (12.0%) had no BCG scar. Of the 35 patients with osteomyelitis, only 5 (14.3%) had bone lesions contiguous to primary skin lesions.

Table 1 shows the results for the entire patient population. The percentage of patients with osteomyelitis and a BCG scar was 12.2, while 35.7% of patients with osteomyelitis did not have a BCG scar. This difference was highly significant ($P = 0.002$). The frequency of osteomyelitis in the entire study population was 15.0%; this compares favorably with the average of 14.8% for the years 1997 to 2001.

The frequency of osteomyelitis in patients with BCG scars was determined in children under 15 years of age (Table 2) and in patients 15 years old or older (Table 3). Among the 139 children under 15 years of age, 130 (93.0%) had a BCG scar and 9 had no BCG scar. The frequencies of osteomyelitis were 33.3% in children without BCG scars and 7.7% in children with BCG scars (Table 2). This difference is statistically significant ($P = 0.039$).

As shown in Table 3, among the 94 patients who were at least 15 years of age, 75 (79.8%) had a BCG scar. The frequencies of osteomyelitis were 36.8% in patients without BCG scars and 20.0% in patients with BCG scars. This difference was not statistically significant ($P = 0.137$).

TABLE 2. Relationship between osteomyelitis and neonatal BCG vaccination scar in children under 15 years of age

BCG scar	No. of patients (%)		Total no. of patients
	With osteomyelitis	Without osteomyelitis	
Present	10 (7.7)[a]	120 (92.3)	130
Absent	3 (33.3)[a]	6 (66.7)	9
Total	13	126	139

[a] The percentages of patients with osteomyelitis with and without BCG scars were statistically significant ($P = 0.039$) by Fisher's exact test.

The sociodemographic characteristics of patients admitted to this study did not vary noticeably during the period of observation. Cases were always found by passive detection. The catchment area of the treatment center remained stable. Gender ratios (Male/female) for those with or without bone lesions were 1.06 and 0.99, respectively, and the median age of both groups was 14 years.

These results show that effective BCG vaccination at birth may protect BU patients against the development of severe disseminated disease (e.g., osteomyelitis). Segregation by age revealed that children under 15 years were better protected than older patients.

The protective efficacy of BCG has been amply demonstrated for prevention of disseminated tuberculosis in children (tuberculous meningitis and miliary tuberculosis) (16). The protective effect of single and booster BCG vaccination has been extensively evaluated (involving 121,020 individuals) in Malawi with a 5- to 9-year follow-up to detect protection against both leprosy and tuberculosis (3). In the Malawi study, "single" or "booster" BCG vaccination status was determined by whether or not the individual had a BCG scar on entry into the study. While a single BCG vaccination gave 50% protection, a second BCG vaccination gave an additional 50% protection for leprosy, but not for tuberculosis. All age groups were included in this study, but protection appeared greatest in those receiving booster vaccinations before 15 years of age.

At this time, BCG vaccination remains the only ethically acceptable intervention for the prevention of BU, especially of the severe forms of the disease in children. We believe that BCG vaccination with one or more booster vaccinations is a valid strategy for trials on the control of BU and should be assessed in large populations at risk for BU. Because BU is distributed focally in rural areas in all countries in Africa in which BU is endemic, populations of children at risk are easily targeted and readily followed after vaccination intervention.

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