INFECTION AND IMMUNITY, Jan. 2004, p. 62–65 Vol. 72, No. 1
0019-9567/04/$08.00+0 DOI: 10.1128/IAI.72.1.62–65.2004

Mycobacterium bovis BCG Vaccination as Prophylaxis against *Mycobacterium ulcerans* Osteomyelitis in Buruli Ulcer Disease

F. Portaels,[1]* J. Aguiar,[2] M. Debacker,[1] A. Guédénon,[3] C. Steunou,[2] C. Zinsou,[2] and W. M. Meyers[4]

Mycobacteriology Unit, Department of Microbiology, Institute of Tropical Medicine, B-2000 Antwerp, Belgium[1]*; Centre Sanitaire et Nutritionnel Gbemoten, Zagnanado,*[2] *and PNLUB, Cotonou,*[3] *Benin; and Armed Forces Institute of Pathology, Washington, D.C. 20306*[4]

Received 3 June 2003/Returned for modification 14 August 2003/Accepted 29 September 2003

Mycobacterium ulcerans **disease, or Buruli ulcer (BU), causes significant morbidity in West Africa. Clinically, the disease presents in the skin as either nonulcerative or ulcerative forms and often invades bones either subjacent to the skin lesion (contiguous osteomyelitis) or remote from the skin lesion (metastatic osteomyelitis). Osteomyelitis represents a severe form of the disease that often requires numerous surgical interventions, even amputations. Surgery is accepted as the present definitive treatment for BU. In the absence of an effective drug treatment, the need for the development of preventive and control strategies becomes paramount. No specific vaccine, however, is presently available for BU. Of 372 consecutive patients in Benin presenting with BU (confirmed by microbiological and histopathological analyses) whose *Mycobacterium bovis* BCG scar statuses were known, 196 children (<15 years old) and 108 adults had neonatal BCG vaccination scars. Of 196 children with BCG scars, 17 (8.7%) had osteomyelitis, while 7 of 28 children without BCG scars (25.0%) had osteomyelitis. Of 108 adults with BCG scars, 17 (15.7%) had osteomyelitis, while 14 of 40 adults without BCG scars (35.0%) had osteomyelitis. Our results show that effective BCG vaccination at birth provides significant protection against the development of *M. ulcerans* osteomyelitis in children and adults. Therefore, health authorities should give attention to the enhancement of neonatal BCG vaccination coverage in all countries of Africa where BU is endemic. Protection against severe forms of BU and childhood tuberculosis would likewise be improved by this intervention.**

Mycobacterium ulcerans disease, commonly called Buruli ulcer (BU), is the third most common mycobacterial disease in humans, after tuberculosis and leprosy (17). Increasing prevalences of BU have been reported in recent years, especially in West Africa (1, 2, 9; M. Debacker, J. Aguiar, C. Steunou, C. Zinsou, W. M. Meyers, A. Guédénon, J. T. Scott, M. Dramaix, and F. Portaels, submitted for publication). In some areas, the incidence of the disease exceeds those of leprosy and tuberculosis (7). Infections are most common in children under 15 years of age.

Although the epidemiology of BU is incompletely understood, foci of BU are closely associated with wetlands, especially slow-flowing or stagnant water in rural settings (12). Transmission to humans may involve water-dwelling insects (8, 14) and other aquatic animals, such as small fish and snails. These animals might also be mechanical reservoirs (13; F. Portaels, P. Elsen, M. Eddyani, A. Martin, W. M. Meyers, and J. T. Scott, unpublished data).

Clinically, BU presents in the skin as either nonulcerative forms (papules, nodules, plaques, and edematous forms) or ulcerative forms (17). The disease is not limited to skin but often invades bone with foci either subjacent to a skin lesion (contiguous osteomyelitis) or remote from the skin lesion (metastatic osteomyelitis) (16). Metastatic bone lesions may develop from the hematogenous or lymphatic spread of *M. ulcerans* from an earlier skin lesion (7).

In Benin, we studied the clinical presentation of 1,611 BU patients who were admitted to the Centre Sanitaire et Nutritionnel Gbemoten at Zagnanado and found that 13.2% of these patients had osteomyelitis (Debacker et al., submitted). Osteomyelitis represents a severe form of the disease that often requires numerous surgical interventions, even amputation.

Of the risk factors for bone involvement in BU, the absence of a neonatal BCG vaccination scar was considered the most significant (16). In 2002, we presented results suggesting that *Mycobacterium bovis* BCG vaccination at birth protected children under 15 years of age against the development of severe disseminated disease (e.g., osteomyelitis) but did not protect adults aged ≥15 years (15). Here we report observations for a larger number of patients for whom diagnosis and treatment took place at the Centre Sanitaire et Nutritionnel Gbemoten.

MATERIALS AND METHODS

A total of 372 consecutive patients with BU that had been confirmed by microbiological analyses (direct smearing for acid-fast bacilli, in vitro culture of *M. ulcerans*, and IS*2404* PCR) and histopathological analyses were studied. Only patients for whom findings were positive for at least two of these four tests were recruited for this study (17). All patients were examined for the presence of a BCG scar by two independent observers. Patients for whom findings were discrepant were excluded from the study. The BCG scar was chosen for the evaluation of a BCG vaccination because it is the most reliable indicator of a successful BCG vaccination (11).

Statistical analysis. The χ^2 test or, when required, the Fisher exact test was used for statistical analysis.

* Corresponding author. Mailing address: Department of Microbiology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. Phone: 32-3-247-63-17. Fax: 32-3-247-63-33. E-mail: portaels@itg.be.

TABLE 1. Relationship between osteomyelitis and neonatal BCG vaccination scars

Population and BCG scar status	No. (%) of patients:		Total no. of patients	*P*
	With osteomyelitis	Without osteomyelitis		
Total patient population				
Present	34 (11.2)	270 (88.8)	304	<0.001
Absent	21 (30.9)	47 (69.1)	68	
Total	55 (14.8)	317 (85.2)	372	
Patients <15 yr old				
Present	17 (8.7)	179 (91.3)	196	0.017
Absent	7 (25.0)	21 (75.0)	28	
Total	24	200	224	
Patients ≥15 yr old				
Present	17 (15.7)	91 (84.3)	108	0.011
Absent	14 (35.0)	26 (65.0)	40	
Total	31	117	148	

RESULTS

Of the 372 BU patients, 304 (81.7%) were found to have BCG scars and 68 (18.3%) had no BCG scars. Table 1 shows the relationship between BCG vaccination and osteomyelitis in the total population studied. Of the BU patients with BCG scars, 11.2% had osteomyelitis, while 30.9% of those without BCG scars had osteomyelitis. This difference is highly significant ($P < 0.001$). The frequency of osteomyelitis in the entire studied population was 14.8%.

The frequency of osteomyelitis in patients with BCG scars was determined for children under 15 years of age and for patients 15 years old or older (Table 1). Of the 224 children under 15 years of age, 196 (87.5%) had BCG scars and 28 did not. The frequencies of osteomyelitis were 8.7% in children with BCG scars and 25.0% in children without BCG scars (Table 1). This difference is statistically significant ($P = 0.017$).

As shown in Table 1, of 148 patients who were at least 15 years of age, 108 (73.0%) had BCG scars. The frequencies of osteomyelitis were 15.7% in patients with BCG scars and 35.0% in patients without BCG scars. This difference is statistically significant ($P = 0.011$).

Gender ratios (male/female) for patients with and without osteomyelitis were 1.04 and 0.99, respectively, and the median age of both groups was 15 years.

There was no statistically significant difference with regard to BCG status between the 14 patients with contiguous bone disease and the 41 patients with metastatic osteomyelitis (data not shown). No significant difference in observed levels of protection could be detected between children under 5 years old, children between 5 and <10 years old, and children between 10 and <15 years old (data not shown).

DISCUSSION

The protective efficacy of BCG vaccination in the prevention of disseminated tuberculosis in children (tuberculous meningitis and miliary tuberculosis) has been amply demonstrated. The protective effects of single and booster BCG vaccinations have been extensively evaluated (in a study involving 121,020 individuals) in Malawi with a 5- to 9-year follow-up to evaluate protection against both leprosy and tuberculosis (6). In the Malawi study, "single" or "booster" BCG vaccination status was established by whether or not an individual had a BCG scar on entry into the study. While a single BCG vaccination gave 50% protection against leprosy, a second BCG vaccination gave an additional 50% protection against leprosy but not against tuberculosis. All age groups were included in the Malawi study, but levels of protection appeared to be greatest in those receiving booster vaccinations before 15 years of age.

In our present study, all age groups were included. Our results show that effective BCG vaccination at birth protects children and adults against the development of *M. ulcerans* osteomyelitis, a severe disseminated disease. A previous study involving 233 BU cases and including only 94 adult patients suggested a protective but insignificant ($P = 0.137$) effect of BCG vaccination against osteomyelitis in adults. Findings for a larger number of adults (148 patients) rendered the protective effect statistically significant.

The sociodemographic characteristics of patients evaluated in this study did not differ from those of patients evaluated in the previous study. All cases were found by passive detection. The demographics and civic affairs of the catchment area of the treatment center remained stable during this period.

The osteomyelitis frequency found in the present study (14.8%) compares favorably with the average of 13.2% (213 of 1,611 patients) for all BU patients treated in the same hospital between 1997 and 2001 (Debacker et al., submitted).

Some risk factors for bone involvement in BU have been identified and include prolonged median delay in presentation at the hospital (16) and concurrent human immunodeficiency virus infection (16). In this study, we have demonstrated that the absence of a BCG scar is an additional risk factor for osteomyelitis in both children and adults with BU. Consideration of these risk factors suggests the following initiatives to reduce the frequency of *M. ulcerans* osteomyelitis and the severe disability associated with the disease: (i) patient presentation delay could be reduced by active early case identification and by promotional actions to increase public awareness (Debacker et al., unpublished data), and (ii) because BCG vaccination at birth protects BU patients against severe disseminated forms of BU such as osteomyelitis, the existing national

BCG vaccination programs in all countries where BU is endemic should be actively promoted.

The control of BU, like most public health problems, involves multiple socioeconomic, environmental, and biomedical issues. The elimination or containment of the etiologic agent depends on favorable environmental alterations. In areas of Benin where BU is endemic, there is a positive correlation between the rates of detection of *M. ulcerans* DNA in the environment and the coincident numbers of BU patients from the same areas (F. Portaels, K. Chemlal, A. Ablordey, M. Debacker, A. Guédénon, P. A. Fonteyne, C. R. Johnson, R. Kotlowski, A. Martin, W. M. Meyers, C. Uwizeye, C. Zinsou, and P. Elsen, unpublished data). The amelioration of socioeconomic conditions such as access to protected sources of water should markedly reduce the transmission of BU (21).

In areas where BU is endemic, neither environmental nor socioeconomic factors are likely to improve in the near future, making prevention by immunoprophylaxis the only logical approach to disease control. Immunoprophylaxis rationales may be directed at the neutralization of the necrotizing toxin elaborated by *M. ulcerans* (3) or the stimulation of a delayed-type hypersensitivity to *M. ulcerans*, such as that achieved by BCG vaccination.

Other proposed candidate vaccines include avirulent *M. ulcerans* strains (P. L. C. Small, T. Alford, and A. Me-Obiang. Abstr. 6th WHO Advisory Group Meet. Buruli Ulcer, abstr., 2003), DNA vaccines (5), and DNA encoding antigen 85A from *M. bovis* BCG (19).

Two prospective trials have shown that BCG vaccination confers rates of protection against BU ranging from 18 to 74%, with an overall protection rate of 47% (18, 20). In addition, vaccinated individuals who develop BU usually have less severe forms of the disease, and histopathologically, the cellular responses are more reactive.

The mode(s) of action for the prophylactic effect of BCG in diminishing the prevalence of severe forms of BU is not well understood. There is abundant histopathologic evidence that BU lesions heal spontaneously by the development of delayed-type hypersensitivity granulomas; however, this is usually a late event in BU disease (10). In active disease, cell-mediated immunity is suppressed, resulting in T-cell anergy to *M. ulcerans* that prevents a Th1-type cellular response in early or active BU disease. Gooding et al. (4) have shown by in vitro studies that prior sensitization to either *M. ulcerans* or BCG promotes the proliferation of peripheral blood mononuclear cells and the production of gamma interferon. Thus, once sensitized to appropriate mycobacterial antigens (e.g., BCG), individuals can mount an effective Th1-type response on exposure to *M. ulcerans*. The necrotizing effect of *M. ulcerans* may limit the exposure of the etiologic agent to immunocompetent cells, permitting the development of local lesions (e.g., cutaneous ulcers); however, when the organisms spread (e.g., to bone), contact with specifically stimulated circulating immunocompetent cells may provide sufficient stimulation of strong cell-mediated immunity of a Th1-type response to prevent clinical metastatic lesions.

Our present results indicate that at this time BCG vaccination remains the only ethically acceptable intervention for the prevention of severe forms of BU. Health authorities should give attention to the enhancement of neonatal BCG vaccination coverage in all countries of Africa where BU is endemic. Protection against severe forms of BU and childhood tuberculosis would likewise be improved by this intervention. Further, the effect of a booster vaccination with BCG should be evaluated. A placebo-controlled field trial of BCG booster vaccinations was recently proposed by the World Health Organization to determine the value of such vaccinations in enhancing protection against all forms of BU (P. D. R. Johnson, Abstr. 6th WHO Advisory Group Meet. Buruli Ulcer, abstr., 2003). An increase in neonatal BCG vaccination coverage and the booster BCG trial have great potential as effective public health interventions for the prevention of BU or some of its disabling features.

ACKNOWLEDGMENTS

This work was supported by the Directorate-General for Development Cooperation, Brussels, Belgium, and by the Damien Foundation, Brussels, Belgium.

We thank K. Janssens and C. Maeckelbergh for preparation of the manuscript.

REFERENCES

1. **Aguiar, J., M. C. Domingo, A. Guédénon, W. M. Meyers, C. Steunou, and F. Portaels.** 1997. L'ulcère de Buruli, une maladie mycobactérienne importante et en recrudescence au Bénin. Bull. Seances Acad. R. Sci. Outre-Mer **3:**325–356.
2. **Amofah, G. K., C. Sagoe-Moses, and E. H. Frimpong.** 1993. Epidemiology of Buruli ulcer in Amanasie, West District, Ghana. Trans. R. Soc. Trop. Med. Hyg. **87:**644–645.
3. **George, K. M., D. Gunawardana, D. Welty, J. Hayman, R. Lee, and P. L. Small.** 1999. Mycolactone: a polyketide toxin from *Mycobacterium ulcerans* required for virulence. Science **283:**854–857.
4. **Gooding, T. M., P. D. R. Johnson, D. E. Campbell, J. A. Hayman, E. L. Hartland, A. S. Kemp, and R. M. Robins-Browne.** 2001. Immune response to infection with *Mycobacterium ulcerans*. Infect. Immun. **69:**1704–1707.
5. **Huygen, K.** 2003. On the use of DNA vaccines for the prophylaxis of mycobacterial diseases. Infect. Immun. **71:**1613–1621.
6. **Karonga Prevention Trial Group.** 1996. Randomized controlled trial of single BCG, repeated BCG, or combined BCG and killed *Mycobacterium leprae* vaccine for prevention of leprosy and tuberculosis in Malawi. Lancet **348:**17–24.
7. **Lagarrigue, V., F. Portaels, W. M. Meyers, and J. Aguiar.** 2000. L'ulcère de Buruli: attention aux atteintes osseuses! A propos de 33 cas observés au Bénin. Med. Trop. **60:**262–266.
8. **Marsollier, L., R. Robert, J. Aubry, J.-P. Saint André, H. Kouakou, P. Legras, A.-L. Manceau, C. Mahaza, and B. Carbonnelle.** 2002. Aquatic insects as vector for *Mycobacterium ulcerans*. Appl. Environ. Microbiol. **68:**4623–4628.
9. **Marston, B. J., M. O. Diallo, R. Horsburgh, S. M. Ostroff, and R. C. Good.** 1995. Emergence of Buruli ulcer disease in the Daloa region of Côte d'Ivoire. Am. J. Trop. Med. Hyg. **52:**219–224.
10. **Meyers, W. M.** 1995. Mycobacterial infections of the skin, p. 291–377. *In* W. Doerr and G. Seifert (ed.), Tropical pathology, 2nd ed., vol. 8. Springer-Verlag, Berlin, Germany.
11. **Pereira, S. M., I. Dourado, M. L. Barreto, S. S. Cunha, M. Y. Ichiara, M. A. Hijjar, J. C. Goes, and L. C. Rodrigues.** 2001. Sensitivity and specificity of BCG scar reading in Brazil. Int. J. Tuberc. Lung Dis. **5:**1067–1070.
12. **Portaels, F.** 1995. Epidemiology of mycobacterial diseases, p. 207–222. *In* M. S. Schuster (ed.), Clinics in dermatology, vol. 13. Elsevier Science Inc., New York, N.Y.
13. **Portaels, F., K. Chemlal, P. Elsen, P. D. R. Johnson, J. A. Hayman, J. Hibble, R. Kirkwood, and W. M. Meyers.** 2001. *Mycobacterium ulcerans* in wild animals. Rev. Sci. Tech. Off. Int. Epizoot. **20:**252–264.
14. **Portaels, F., P. Elsen, A. Guimaraes-Peres, P.-A. Fonteyne, and W. M. Meyers.** 1999. Insects in the transmission of *Mycobacterium ulcerans* infection (Buruli ulcer). Lancet **353:**986.
15. **Portaels, F., J. Aguiar, M. Debacker, C. Steunou, C. Zinsou, A. Guédénon, and W. M. Meyers.** 2002. Prophylactic effect of *Mycobacterium bovis* BCG vaccination against osteomyelitis in children with *Mycobacterium ulcerans* disease (Buruli ulcer). Clin. Diagn. Lab. Immunol. **9:**1389–1391.
16. **Portaels, F., C. Zinsou, J. Aguiar, M. Debacker, E. de Biurrun, A. Guédénon, R. Josse, V. Lagarrigue, M. T. Silva, C. Steunou, and W. M. Meyers.** Les atteintes osseuses dans l'ulcère de Buruli: à propos de 73 cas. Bull. Séances Acad. R. Sci. Outre-Mer, in press.
17. **Portaels, F., P. Johnson, and W. M. Meyers (ed.).** 2001. Buruli ulcer. Diag-

nosis of *Mycobacterium ulcerans* disease. A manual for health care providers. WHO/CDS/GBUI/2001.4:42p. World Health Organization, Geneva, Switzerland.
18. **Smith, P. G., W. D. L. Revill, E. Lukwago, and Y. P. Rykushin.** 1976. The protective effect of BCG against *Mycobacterium ulcerans* disease: a controlled trial in an endemic area of Uganda. Trans. R. Soc. Trop. Med. Hyg. **70:**449–457.
19. **Tanghe, A., J. Content, J.-P. Van Vooren, F. Portaels, and K. Huygen.** 2001. Protective efficacy of a DNA vaccine encoding antigen 85A from *Mycobacterium bovis* BCG against Buruli ulcer. Infect. Immun. **69:**5403–5411.
20. **Uganda Buruli Group.** 1969. BCG vaccination against *Mycobacterium ulcerans* infection (Buruli ulcer). Lancet **i:**111–115.
21. **Watts, J.** 2003. Forum debates private sector role in global water supply. Lancet **361:**1022–1023.

Editor: S. H. E. Kaufmann