Bacillus Calmette–Guérin immunization in infants born to HIV-1-seropositive mothers

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During the prospective follow-up of 64 babies at risk for perinatal HIV-1 infection because their mothers were seropositive, and of 130 control babies whose mothers were seronegative, we studied the occurrence of complications of bacillus Calmette–Guérin (BCG) immunization and its ability to induce cutaneous reactivity to tuberculin. Babies born both to HIV-1-positive and HIV-1-negative mothers received BCG immunization during their first month of life according to the Expanded Programme on Immunization (EPI) recommendations. Local and regional complications of BCG vaccine were looked for at 3, 6 and 9 months after inoculation. A tuberculin skin test was performed at 6 or 9 months of age. Most babies born to HIV-1-positive mothers were later classified as infected or uninfected according to their clinical condition and/or serological status at 18 months of age. The mean duration of the follow-up was 36 months (range 30–40 months). No chronic or deep ulcerations at the site of injection or disseminated forms of BCG infection were observed. The frequency of BCG-related lymphadenitis in the group of HIV-1-infected children (24%) did not differ significantly from the group of uninfected children (19%; Fisher test: $P = 0.73$). In contrast, the tuberculin skin test responses were positive less often in the group of HIV-1-infected children (33%) than in the uninfected group (83%; Fisher test: $P = 0.007$). Because BCG vaccine appears to be safe — even when given to perinatally infected babies — continuation of the BCG immunization policies of the EPI is justified, especially in view of the growing incidence of tuberculosis as a complication of HIV infection.

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Introduction

Tuberculosis is of major public health importance in Africa and is becoming an even greater problem because of its exacerbation by the HIV epidemic [1,2]. In areas were tuberculosis is endemic, bacillus Calmette–Guérin (BCG) immunization at birth is still the basic method for tuberculosis prevention [3–5]. Fatal complications of BCG immunization have been reported in infants with congenital immunodeficiency [6,7], and case reports have raised doubts about the safety of BCG in HIV-1-infected infants because of their impaired immunity [8–13]. In addition, the presence of HIV-related immunodeficiency might decrease the response to tuberculin antigen and the accompanying protective effect of BCG immunization [14,15].

To date, no published study in infants has given prospective data concerning the effect of BCG immunization in the setting of HIV infection. The present investigation was designed within an established prospective cohort study on mother–child transmission of HIV infection [16]. The objective of our investigation was to evaluate both the occurrence of locoregional complications of BCG inoculation at birth, and the induction of subsequent tuberculin reactivity in HIV-1-infected and uninfected infants born to seropositive mothers and in control infants born to seronegative mothers.

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Subjects and methods

Pregnant women were recruited sequentially, after giving informed consent, during their first prenatal visit to one of two mother–child clinics serving the eastern districts of Brazzaville, Congo. Sera were tested for antibodies to HIV-1 by enzyme-linked immunosorbent assay (ELISA; ELAVIA, Diagnostics Pasteur, Marnes-La-Coquette, France). Positive ELISA tests were confirmed by Western blot (Blot test, Du Pont de Nemours, Wilmington, Delaware, USA). Sera were considered positive when they showed antibodies to at least two envelope glycoproteins of HIV-1.

Each seropositive woman was individually matched for age, estimated date of delivery, and place of residence (district) with two seronegative pregnant women. After birth, infants were followed with clinical examination and blood sampling by the same physician at 1 month, 3 months, and every 3 months thereafter (active follow-up included home visit for individuals not presenting for scheduled visits). The mean duration of the follow-up was 36 months (range 30–40 months).

Babies born to both seropositive and negative mothers received BCG immunization (Pasteur Vaccins, strain 1173P2, Diagnostics Pasteur) during their first month of life according to the World Health Organization (WHO) recommendations: 0.05 mg in 0.05 ml via intradermal injection on the lateral surface of the left arm [17].

The infants were examined for local and regional complications of BCG immunization at 3, 6 and 9 months after inoculation. Isolated left axillary and/or supraclavicular lymph nodes >1 cm in diameter, without other discernible local cause, were considered to be related to BCG.

A tuberculin skin test (Monotest, Mérieux, Lyon, France; intradermal route by multipuncture of tuberculin antigen at a dose of 300 000 IU/ml) was performed at 6 or 9 months of age. The delayed hypersensitivity response to tuberculin was reported by the same physician 48 h later. A positive skin reaction was defined as an area of induration with a diameter >2 mm [18].

The 178 babies on whom follow-up studies are available were subdivided into four groups. Group 1: HIV-1-infected infants who had AIDS according to the WHO clinical surveillance definition and/or who were seropositive at 18 months of age or older; group 2: HIV-1-indeterminate infants whose clinical status did not fit the AIDS definition or who were younger than 18 months; group 3: HIV-1-uninfected infants, i.e. those who were seronegative after 18 months of age, and group 4: infants born to seronegative mothers.

Statistical analyses (χ^2 test, Fisher's exact two-tailed test, and Student's t-test) were performed using BMDP Statistical Software [19]. Statistical significance was designated at $P < 0.05$.

Results

Fifty-two of the 64 infants born to seropositive mothers (81%) and 127 out of 130 infants born to seronegative mothers (98%) were vaccinated by BCG and could be traced for more than 3 months. The other babies were either lost to follow-up (one born to a seropositive mother and one to a seronegative) or had died (11 had seropositive and two had seronegative mothers). Causes of death are shown in Table 1.

Table 1. Causes of death among the infants before 6 months of age.

	Mothers	
Cause of death	HIV+	HIV−
Prematurity or hypotrophy	5	2
AIDS	6	0
Acute respiratory infection	5	0
Unknown cause	2	1
Total	18	3

No chronic or deep ulcerations were observed at the site of injection of BCG. The frequency of BCG-related lymphadenitis (Table 2) was similar in infants born to seropositive and to seronegative mothers: 21% (11 out of 52) versus 18% (23 out of 127), respectively ($\chi^2 = 0.22$, not significant). Among the infants born to seropositive mothers, the frequency of BCG-related lymphadenitis was similar in HIV-1-infected infants (group 1) to that in uninfected infants (group 3): 24% (five out of 21) versus 19% (five out of 27; Fisher test, $P = 0.73$).

Table 2. Frequency of bacillus Calmette–Guérin-related adenitis according to HIV-1 status of infant.

	Group 1 infants n (%)	Group 2 infants n (%)	Group 3 infants n (%)	Group 4 infants n (%)
Lymphadenitis present	5 (24)	1 (25)	5 (19)	23 (18)
Lymphadenitis absent	16 (76)	3 (75)	22 (81)	104 (82)
Total	21 (100)	4 (100)	27 (100)	127 (100)

Group 1 infants, infants with AIDS or HIV-1-seropositive at 18 months of age; group 2 infants, HIV-1-indeterminate infants of HIV-1-infected mothers; group 3 infants, HIV-1-uninfected infants of HIV-1-infected mothers; group 4 infants, HIV-1-uninfected infants of uninfected mothers. Group (1 + 2 + 3) versus (4), $\chi^2 = 22$, $P = 0.63$ (NS); group (1) versus (3), Fisher exact test $P = 0.73$ (NS); group (1) versus (3 + 4), Fisher exact test $P = 0.55$ (NS).

Lymphadenitis, including one case with a fistula in an infant born to a seronegative mother, resolved spontaneously in all instances.

No disseminated forms of BCG infection were detected during the follow-up period.

Results of the tuberculin skin tests were available for 36 infants born to seropositive mothers (56%) and 105 infants born to seronegative mothers (81%). The others were lost to follow-up (four infants born to seropositive

Table 3. Frequency of positive tuberculin skin test (>2 mm) according to HIV-1 status of infant.

	Group 1 n (%)	Group 2 n (%)	Group 3 n (%)	Group 4 n (%)
Skin test >2 mm	4 (33)	0 (0)	19 (83)	88 (84)
Skin test ≤2 mm	8 (67)	1 (100)	4 (17)	17 (16)
Total	12 (100)	1 (100)	23 (100)	105 (100)

Groups 1–4 as in Table 2. Group (1+2+3) versus (4), $\chi^2 = 6.35$, $P = 0.01$; group (1) versus (3), Fisher exact test $P = 0.007$; group (1) versus (3 + 4), Fisher exact test $P = 0.0004$.

mothers and six born to seronegative mothers), did not have their skin tests read (six with seropositive and 16 with seronegative mothers), or had died before the age of 6 months (18 and three, respectively).

The frequency of positive skin tests (Table 3) was lower in infants born to HIV-1-seropositive mothers than in infants born to seronegative mothers: 64% (23 out of 36) versus 84% (88 out of 105; $\chi^2 = 6.35$; $P = 0.01$). Among the infants born to seropositive mothers, the frequency of positive tuberculin tests was even lower among HIV-1-infected infants (group 1) than in uninfected infants (group 3): 33% (four out of 12) versus 83% (19 out of 23; Fisher test: $P = 0.007$).

The mean diameter of induration was 4.0 mm (s.d. = 2.76) among infants born to seropositive mothers and 5.0 mm (s.d. = 2.56) among infants born to seronegative mothers (t-test: 1.96, $P = 0.052$). It was significantly lower in HIV-1-infected infants (group 1) than in uninfected infants (group 3): 2.0 mm (s.d. = 1.86) versus 5.1 mm (s.d. = 2.51), respectively (t-test: 3.80, $P = 0.0001$). Distributions of the induration responses are shown in Fig. 1.

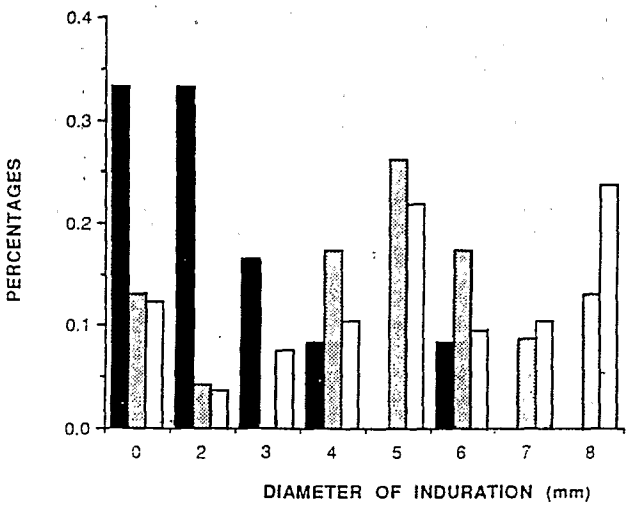

Fig. 1. Distribution of the tuberculin skin test responses according to HIV-1 status of infant. ■, Group 1 infants (infants with AIDS or HIV-1-seropositive at 18 months of age); ▨, group 3 infants (HIV-1-uninfected infants of HIV-1-infected mothers); □, group 4 infants (uninfected infants born to HIV-1-seronegative mothers).

Discussion

The results from this study show that there was no significant difference in the frequency of BCG-related lymphadenopathy either in babies born to HIV-1-seropositive or seronegative mothers, or in babies with or without HIV-1 infection. In contrast, babies with HIV-1 infection had less cutaneous reactivity to tuberculin than babies without HIV-1 infection. These results provide further insight into discussions concerning the risks versus benefits of BCG immunization as currently recommended by the Expanded Programme on Immunization (EPI) in high HIV prevalence areas [17].

The high frequency (18%) of regional lymphadenitis in the overall study population, which is higher than previously reported by the Brazzaville Centre for Tuberculosis Control [20], could be attributable to several factors: (1) the BCG strain used for immunization is known as a 'hot' strain by comparison with the Glaxo or Tokyo strain [21]; (2) the systematic and active follow-up of the present cohort of vaccinated infants, and (3) the criteria for the diagnosis of lymphadenitis (size ≥1 cm), which is less stringent than in other studies where only cases of abscessed lymphadenitis are considered [22].

The similar frequency of lymphadenitis after BCG inoculation between infants born to seropositive and seronegative mothers is consistent with the results of studies carried out using a longitudinal approach in Zaire [23], Kenya [24] and Uganda [25], although the HIV status of the babies was not shown. In the present study, the HIV status was known for most infants, and this infection was not associated with a significantly higher frequency of locoregional complications and dissemination was not detected.

Serological assessment of 19 babies who were evaluated in Kinshasa [22] for BCG-related abscess revealed that all were HIV-negative. This finding also emphasizes the absence of a relationship between HIV serological status and complications of BCG immunization. Five cases of suppurative BCG-related adenitis in HIV-1-infected babies have been observed in hospitals in France (Griscelli C, Blanche S, personal communication), but the frequency of this complication among all BCG-vaccinated babies cannot be evaluated from such uncontrolled reports.

Some cases of disseminated BCG infection have been reported in HIV-1-infected adults [26,27]. One case has been described in an HIV-infected infant from Zaire [13]. The fact that no babies with disseminated BCG infection were observed in the Brazzaville study after a mean follow-up of 36 months is in agreement with the results of similar prospective studies carried out in Zaire [23] and Kenya [24]. The estimated risk of disseminated BCG infection among babies with severe congenital immunodeficiency is 1% [28]. If one assumes that the same high risk of dissemination might be seen in HIV-1-related immunodeficiency, sufficiently large cohort studies are needed in order to detect cases of disseminated BCG infection [23,24]. In the absence of radiological and bacteriological investigations and of postmortem verification,

it is impossible to exclude the occurrence of disseminated BCG in the children in the present study who died. The circumstances of their deaths, however, do not support this diagnosis. As is the case for many HIV-related opportunistic infections, a possible reactivation of BCG may occur as a late complication of the profound immunological deterioration accompanying HIV infection. Accordingly, there is a need for additional investigations into the cause of death in HIV-positive babies who die after BCG vaccination.

In the present study, the fact that HIV-1 infection in infants was associated with a lower frequency of positive tuberculin tests suggests the early occurrence of immunodeficiency in infected infants. The percentage of negative tuberculin tests among infants might have been underestimated, since several of these infants died of conditions related to HIV-1 immunodeficiency before they were skin-tested, and they most likely would have scored as tuberculin-negative. Nevertheless, 33% of the HIV-1-infected infants in whom the response to tuberculin was evaluated did develop a positive test and, therefore, might have achieved a level of protection against serious complications of primary tuberculosis [29,30].

In HIV-endemic areas in which BCG immunization is utilized, the HIV-1 serostatus of a mother may be known. In this setting, given the particularly high risk of tuberculosis for infants (HIV-1-infected or uninfected) and the likelihood of an HIV-seropositive mother having tuberculosis, BCG immunization can be safely performed except in symptomatic infants. The feasibility of routine HIV-1 screening in pregnant women is low virtually everywhere in Africa [31], and the diagnosis of HIV-1 infection in neonates is not practical [32]. Therefore, immunization strategies can be planned only at a population level. From the results of the present study, we conclude that the EPI policies on BCG immunization [33] should be reinforced in areas where HIV-1 infection is highly prevalent because: (1) BCG immunization is safe, irrespective of the serological status of mothers and infants; (2) the risk of tuberculosis is greatly increased in regions such as subSaharan Africa where there is a high prevalence of both tuberculosis and HIV infection [34–38]; (3) the protection conferred by BCG immunization to uninfected babies born to seropositive mothers far outweighs the theoretical risk of disseminated infection in HIV-infected babies, and (4) some HIV-infected children (those who develop a positive tuberculin skin test) may be transiently protected from serious complications of primary tuberculosis.

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