A Pilot Study of Three Potential Vaccines for Leprosy in Bombay¹

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Trials of the efficacy of BCG vaccination against leprosy have produced variable results, but the study carried out in Uganda showed that under some conditions at least it could be very effective (2). The development of soluble preparations of leprosy bacilli for skin testing (4, 14) has meant that modified vaccines could be assessed without the expense of direct protection trials, on the assumption that the induction of positivity to these skin-test reagents indicates an improved level of immune protection. Thus, Convit and his colleagues have used their soluble leprosy reagent (4) to assess the effects of a combination of BCG plus killed Mycobacterium leprae as a vaccine (3). This vaccine is currently under trial as to its direct protective effect in South America and Malâwi.

The soluble reagent leprosin A, prepared in London (¹⁴) from leprosy bacilli, has been used to assess the effects of BCG in the Indian towns of Agra (¹⁰) and Ahmednagar (¹⁵), and to evaluate the combinations of BCG with *M. vaccae* or *M. leprae* in The Lebanon (¹) and in Iran (^{5, 16}). In the present study, we have used this reagent together with three other new tuberculins (°) to determine the effects of these combinations in children attending schools in the slums of Bombay, India. Bombay is an enormous city with 8–9 million inhabitants where both leprosy and tuberculosis are endemic. In the slums where the children live environmental mycobacteria abound, and even the piped drinking water may contain up to 10^8 mycobacteria per liter (⁶).

MATERIALS AND METHODS

Reagents used. The skin-test reagents used were four of the series of new tuberculins (9)-tuberculin, leprosin A, scrofulin, and vaccin-prepared from M. tuberculosis, M. leprae. M. scrofulaceum, and M. vaccae, respectively. They were given as 0.1 ml intradermal injections at least 10 cm apart two on each forearm. The doses injected were 0.2 μ g of tuberculin and scrofulin, 1.0 μ g of leprosin A, and 2.0 μ g of vaccin, as used in previous studies (1, 5, 16). Diameters of induration were measured after 72 hr. and mean reaction sizes of 2 mm or more were considered positive. Our experience in other studies (5, 10, 14-16) led us to take this small size as positive. Proper use of the short bevel, 26-gauge, intradermal needle, which causes minimal trauma (rarely more than 1×1 mm) has not led to problems in the interpretation of small-sized reactions. The arbitrary decision that 4×5 mm is a negative response and 5 \times 5 mm a positive response has never seemed sensible to us, especially when biopsy data show that the same cells infiltrate very small reactions (and a proportion of zero reactions) as infiltrate large reactions (17).

The vaccines used were the same as those previously employed in studies in The Lebanon $(^1)$, and in Iran $(^{5, 16})$. BCG Glaxo (freeze

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	Vaccine A	Vaccine B	Vaccine C	
Tuberculin				
Time 0	17/64 (27%) 3.24 ± 1.00 mm p < 0.00001 ^b	$\begin{array}{l} 18/61 \ (30\%) \\ 2.90 \ \pm \ 0.71 \ \mathrm{mm} \\ \mathrm{p} \ < \ 0.00001 \end{array}$	17/61 (28%) 2.94 ± 0.93 mm p < 0.00001	
1-3 years	49/64 (77%) 12.74 ± 5.90 mm	44/61 (72%) 12.49 ± 7.51 mm	51/61 (84%) 12.04 ± 6.92 mm	
Leprosin A				
Time 0	19/64 (30%) 4.19 ± 3.49 mm NS ^c	$\begin{array}{l} 10/61 \ (16\%) \\ 4.00 \ \pm \ 2.60 \ \mathrm{mm} \\ \mathrm{p} \ < \ 0.002 \end{array}$	19/61 (31%) 4.53 ± 3.76 mm NS	
1-3 years	22/64 (34%) 6.38 ± 5.03 mm	26/61 (43%)24/61 (39%)4.92 ± 3.27 mm5.62 ± 3.76 m		
Scrofulin				
Time 0	36/64 (56%) 5.35 ± 3.93 mm NS	27/61 (44%) 4.52 ± 2.30 mm NS	29/61 (48%) 5.62 ± 3.90 mm NS	
1-3 years	35/64 (55%) 4.92 ± 3.86 mm	30/61 (49%) 5.77 ± 3.85 mm	35/61 (57%) 6.40 ± 3.80 mm	
Vaccine				
Time 0	29/64 (45%) 4.39 ± 2.33 mm NS	26/61 (43%) 4.74 ± 2.46 mm NS NS 25/61 (41%) 4.89 ± 2.84 mm NS		
1-3 years	22/64 (34%) 5.30 ± 4.33 mm	22/61 (36%) 30/61 (49%) 4.74 ± 2.63 mm 5.64 ± 3.33 mm		

TABLE 1. Results of skin tests [number positive/total (% positive), mean \pm S.D. induration] immediately before and 1-3 years after vaccination with BCG alone (vaccine A), BCG plus 10⁷ killed M. vaccae (vaccine B), or BCG plus 10⁷ killed M. leprae (vaccine C).^a

^a Mean age of the children was 7.2 years prior to vaccination.

^b Probabilities calculated between 0 time and 1-3 years by Fisher's exact test.

 $^{\circ}$ NS = difference between 0 time and 1–3 years not statistically significant.

dried) was made up with the water provided by the manufacturer (vaccine A), or the same BCG was made up with a suspension of 108/ ml of M. vaccae in saline (vaccine B), or with a suspension of 10^8 /ml of *M. leprae* in saline (vaccine C). The organisms in these suspensions had been killed by exposure to 2.5 megarads from a 60 cobalt source. Vaccines were administered by an intradermal injection of 0.1 ml high over the left deltoid muscle.

Study plan. The children studied were aged 3-17 years and attended a number of schools in several different slum districts of the city. They were arbitrarily selected for vaccination on the basis of the lack of a scar from earlier BCG vaccination and a skintest reaction to tuberculin of less than 5-mm mean diameter of induration. Out of 1595 children tested, 395 were suitable for vaccination, and 292 were vaccinated - 96 were given vaccine A, 99 were given vaccine B, and 97 were given vaccine C. Among the 1200 children who were not suitable for

vaccination, 775 had BCG scars (523 or 67% of them were tuberculin positive) (11), and 425 had responses to tuberculin of 5 mm or more despite their lack of a BCG scar.

Follow-up skin testing with the four reagents was carried out 1 to 3 years after vaccination in as many children as possible, without the person doing the tests knowing which child had received which vaccine. Thus, the results were recorded "blind." The sizes of the vaccine scars were also measured at these times. Most of the skin testing and vaccinating for the study was carried out by a series of students in the course of their elective studies.

RESULTS

Effects of the three vaccines. The mean age at the time of vaccination of the children who were followed up was 7.2 \pm 1.7 (mean \pm S.D.) years (range 3–15 years), and the mean age of those among them who had responses to leprosin A at the time of vac-

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TABLE 2. Percentages of persons, mainly children, converting to leprosin A positivity after BCG or BCG plus M. vaccae, and the differences between them.

	BCG	Difference	BCG + M. vaccae
Leprosy	-endemic situat	ions	
Iran			
Children of patients (16)	63%	(31%)	94% (8 years) ^a
Village children (⁵)	33%	(16%)	49% (8 years)
India			
Bombay children (this study) ^b	27%	(14%)	41%
Contacts of patients in South Indiac	47%	(12%)	59% (1 year)
Situations n	on-endemic for	leprosy	
Iran			
Village children (15)	53%	(3%)	56% (8 years)
Lebanon			
Village children (¹)	12%	(7%)	19% (3 years)

^a The time in years is the period between vaccination and follow-up skin tests.

^b To make them comparable with data from other studies, percentages shown from the present study are those for children who had zero responses to leprosin A prior to vaccination.

e Preliminary results from an ongoing study.

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cination was 7.0 \pm 1.6 years (range 5–13 years). The skin-test results for these children before and from 1–3 years after vaccination with each of the three vaccines are shown in Table 1. It can be seen that all three vaccines significantly increased tuberculin positivity (p < 0.00001), and that only vaccine B increased leprosin A positivity (p < 0.002). There were no significant effects on responsiveness to scrofulin or vaccin.

The postvaccination scar sizes were the same for all three vaccines, and the mean scar size for all children was 6.1 ± 2.5 mm; two children had no visible scar, and the largest scar measured 17 mm. There were no significant differences between the different schools in the effects of the vaccines or between the scar sizes (data not shown).

DISCUSSION

All three vaccines very significantly increased tuberculin positivity (p < 0.00001) to a similar degree, showing that the additions did not adversely affect tuberculin conversion. The most important observation of the study is that the incorporation of *M. vaccae* with BCG (vaccine B) produced a significant enhancement of postvaccination leprosin-A positivity, which was not seen after BCG alone or after the combination of BCG with *M. leprae*. However, it has to be noted that there was no significant difference in the final percentage positivity to leprosin A after each of the three vaccines. Inclusion of an unvaccinated group in our study might have been desirable, but was not considered ethical.

Leprosy is highly endemic for the slum areas where the schools were situated, and the source of sensitization to leprosin A is likely to be from casual contact with leprosy patients, rather than a direct effect of the vaccine. Thus, the data obtained in urban Bombay support the observations made in rural Iranian Azerbaijan (^{5, 16}) that the combination of BCG Glaxo with *M. vaccae* may be a better vaccine for leprosy than is BCG alone (Table 2). Although the numbers vaccinated were small, it is interesting to note that the addition of *M. leprae* to BCG did not significantly improve recognition of leprosin A over the period of the study.

The mechanism of action of vaccines containing BCG is interesting in that postvaccination positivity directly caused by the vaccine wanes very considerably within 3 years unless boosted from the environment. This is clearly seen in the comparison between results obtained in The Lebanon (¹), where contact with environmental mycobacteria appears to be minimal, and those obtained in Agra (¹⁰) and Ahmednagar (¹⁵), where environmental mycobacteria abound. BCG acts by priming the individual to recognize small environmental challenges and to develop delayed-type hypersensitivity, which may be directed to antigens of group i (common mycobacterial), group iv (^{7, 12, 13}) (species specific), or even of group ii (⁸) (slowgrower associated antigens).

M. vaccae was specifically added to BCG to improve recognition of casually encountered organisms, not to induce positivity to leprosin A by some crossreactivity in species-specific (group iv) antigens between it and the leprosy bacillus. The present study is one of a series in which the effects of the addition of *M. vaccae* are being assessed in situations of differing leprosy endemicity. The results of studies completed or still in progress are shown in Table 2. In each of the studies in leprosy-endemic regions, skintest recognition of the leprosy bacillus has been successfully enhanced.

SUMMARY

Three vaccines, BCG Glaxo alone (vaccine A), BCG Glaxo plus 107 killed Mycobacterium vaccae (vaccine B), and BCG Glaxo plus 107 killed M. leprae (vaccine C). were given to groups of selected children. The effects of these vaccines on subsequent quadruple skin testing 1-3 years after vaccination were compared. All three vaccines equally and significantly (p < 0.00001) increased positivity to tuberculin, but only vaccine B was found to significantly enhance development of skin-test positivity to leprosin A (p < 0.002). The data support the evidence previously obtained in rural Iran that the combination of BCG with killed M. vaccae is likely to be a better vaccine for leprosy than is BCG alone.

RESUMEN

Se seleccionaron 3 grupos de niños a los cuales se les administró la vacuna BCG Glaxo sola (vacuna A), BCG Glaxo más 10⁷ *Mycobacterium vaccae* muertos (vacuna B), o BCG Glaxo más 10⁷ *M. leprae* muertos (vacuna C). Se compararon los efectos de estas tres vacunas sobre las respuestas intradérmicas de los niños a 4 antígenos de prueba, 1–3 años después de la vacunación. Las tres vacunas incrementaron significativamente (p < 0.00001) la reactividad a la tuberculina, pero sólo la vacuna B incrementó significativamente la reactividad a la leprosina A (p < 0.002). Los datos apoyan la evidencia obtenida previamente en Irán de que la combinación de BCG con *M. vaccae* muerto es probablemente una mejor vacuna contra la lepra que el BCG sólo.

RÉSUMÉ

On a administré à des groupes sélectionnés d'enfants trois vaccins, à savoir le BCG Glaxo seul (vaccin A), le BCG Glaxo accompagné de 107 Mycobacterium vaccae tués (vaccin B) et le BCG Glaxo plus 107 M. leprae tués (vaccin C). On a comparé les résultats d'une épreuve cutanée quadruple, pratiquée 1 à 3 ans après la vaccination, dans les trois groupes. Les trois vaccins ont, tous, eu comme effet d'accroître la réaction positive à la tuberculine, de manière statistiquement significative (p < 0.00001). Néanmois, seul le vaccin B renforcait significativement le développement d'une positivité cutanée à la léprosine A (p < 0.002). Ces données confirment les résultats obtenus précédemment dans les régions rurales de l'Iran, qui montraient qu'une combinaison de BCG et de M. vaccae tué paraît constituer un meilleur vaccin pour la lèpre que le BCG seul.

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