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Sensitization Studies with Potential Leprosy Vaccine Preparations in Northern Malawi[1]

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Evidence that BCG vaccines can impart significant if variable protection against clinical leprosy has demonstrated that vaccines may be of use in leprosy control ([7]). The recent availability of purified bacilli and specific antigens from *Mycobacterium leprae* has led to increased hope of a new generation of effective antileprosy vaccines ([2]). Among the central questions currently at issue is whether a vaccine made up of killed *M. leprae* or a combination of killed *M. leprae* and BCG might prove more effective than BCG alone in protecting against leprosy. The only way to assess the protective efficacy of a new vaccine is in the context of a controlled field trial. Given the time and expense of such trials, preliminary studies are necessary not only to assess the safety of potential vaccine preparations but also to demonstrate their ability to induce some sort of cell-mediated "immune response" against antigens from *M. leprae*. Several such studies have already been carried out (Table 1).

The most extensive work has been performed in Venezuela by Dr. J. A. Convit and his colleagues, only a small example of which is given in Table 1 ([3, 4]). Their choice of a combination of BCG and killed *M. leprae* as a prophylactic vaccine arose in the course of testing the potential therapeutic effect of such preparations in multibacillary patients. These workers have shown that intradermal injection of 6×10^8 heat-killed *M. leprae* in combination with BCG (Pasteur strain, 0.2 mg in individuals considered tuberculin "negative" and 0.04 mg in individuals considered tuberculin "positive") induces skin test sensitivity to a *M. leprae* soluble antigen [prepared at the Instituto Nacional de Dermatologia (IND) in Caracas] in a high proportion of individuals initially classed as skin test negative. This skin test sensitivity is quantitatively greater, and of longer duration, than sensitivity to the same skin tests induced by BCG alone. On the basis of these results, Convit and his colleagues have recently begun a large controlled trial of the efficacy of a combined BCG and heat-killed *M. leprae* vaccine in protecting against leprosy.

A small study in Malaysia by Smelt, *et al.* indicated that 2×10^8 radiation-killed *M. leprae* alone or in combination with BCG (Japanese strain, 1.5×10^6 bacilli) was superior to BCG alone in eliciting skin test sensitivity to a *M. leprae* soluble antigen prepared at the National Institute of Medical Research (NIMR) in London (batch AB/22) ([12]).

Another study, by Samuel, *et al.*, compared the sensitizing effects of 10^7 killed *M. leprae* and 10^7 killed *M. vaccae*, each alone and in combination with BCG (Glaxo, 10^6 organisms per dose) among school children in Nepal ([11]). Sensitivity was assessed in terms of a soluble antigen prepared from *M. leprae* at the NIMR in London ("Leprosin A"). Their results indicated that killed *M. leprae* was marginally superior to killed *M. vaccae*, and that the addition of BCG added little if anything to the sensitizing ability. They reported a fall in sensitivity in all groups between skin tests carried out at three months and eight months after vaccination.

Gill, *et al.* have recently carried out a study comparing different doses of radiation-killed *M. leprae* (from 1.5×10^7 to 5×10^8) in eliciting skin test sensitivity among nurses in Norway ([9]). No effect was noted on tuberculin (RT23) sensitivity, but a dose–response relationship was found in terms of sensitivity to a (NIMR-produced) *M. leprae* soluble antigen.

All of these studies agree in demonstrat-

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TABLE 1. *Review of published reports on skin test conversion associated with various leprosy "vaccines."*

Author(s)	Population	Vaccines	Skin test "conversions" among prior "negatives"		
Convit [4]	Venezuela	Combined vaccines:	WEL-1 antigen (≥10 mm at 48 hr = +)	IND antigen (≥10 mm at 48 hr = +)	RT-23 tuberculin (≥10 mm at 48 hr = +)
	Contacts, with BCG scars				
	RT-23 <10 mm	6×10^8 KML[a] + 0.2 mg BCG	17/30 at 12 wks	26/26 at 12 wks	33/39 at 12 wks
	RT-23 ≥10 mm	6×10^8 KML + 0.04 mg BCG			
Gill, *et al.* [9]	Norway (with BCG scars)		NIMR/London-type MLSA[c] (CD-19) Not analyzed in terms of "conversions," but clear dose response in increased MLSA sensitivity 3 months after vaccination	RT-23 tuberculin	
	Nurses	1.5×10^7 KML		No effect	
	Nurses	5×10^7 KML		No effect	
	Nurses	1.5×10^8 KML		No effect	
	Nurses	5×10^8 KML		No effect	
Samuel, *et al.* [11]	Nepal		NIMR/London-type MLSA at 3 mos. (≥5 mm = +)	NIMR/London-type MLSA at 8 mos. (≥5 mm = +)	
	BCG scar status?				
	School children	10^7 KML	98/150 (65%)	54/170 (32%)	
	Contacts	10^7 KML + BCG (1×10^6)	38/55 (69%)	34/55 (62%)	
	School children	10^7 KMV[b]	88/150 (59%)	22/170 (13%)	
	Contacts	10^7 KMV + BCG (1×10^6)	22/60 (37%)	13/60 (22%)	
Smelt, *et al.* [12]	Malaysia		NIMR/London-type MLSA (AB22) at 6 wks (≥5 mm = +)	RT-23 tuberculin (10 IU at 6 wks (≥5 mm = +)	
	17–41 yrs of age				
	BCG scar status?				
	Healthy females	2×10^8 KML	6/7	0/7	
	Healthy females	2×10^8 KML + BCG (1.5×10^6)	7/8	8/8	
	Healthy females	Japanese BCG (1.5×10^6)	2/8	8/8	

[a] KML = killed *M. leprae.*
[b] KMV = killed *M. vaccae.*
[c] MLSA = *M. leprae* soluble antigens.

ing that killed *M. leprae,* either alone or in combination with BCG, can induce sensitivity to skin tests consisting of soluble antigens of *M. leprae.* But they provide little data on the side effects of the vaccines and on the implications of different doses of *M. leprae* in combination with BCG. The study reported in this paper was designed to provide detailed information on these issues as a contribution to discussions of the potential usefulness of such preparations as vaccines for the prevention of leprosy.

The study was carried out in the context of the Lepra Evaluation Project, a large longitudinal study in northern Malawi, with the agreement of the Malawian Ministry of Health.

MATERIALS AND METHODS

The following soluble skin test antigens were used in the study:

RT-23 tuberculin, 2 international units (per 0.1 ml), purchased from the Statens Serum Institute, Copenhagen, Denmark.

WEL-1—a soluble antigen prepared from purified *M. leprae* by Wellcome Laboratories, according to a protocol provided by Dr. J. A. Convit. Preparation of this antigen involved disruption of radiation-killed *M. leprae* in a French press, passage through 0.45 μm millipore and Amicon PM-30 membranes, and autoclaving. Its final concentration was 0.5 μg protein per dose (0.1 ml). This antigen was provided by IMMLEP, and was shipped to Malawi on dry ice.

CD-19—a soluble antigen prepared from purified *M. leprae* at the NIMR in London, following a protocol devised by Dr. R. J. W. Rees ([12]). Preparation of this antigen involved disruption of purified radiation-killed bacilli by sonication, and dilution to 1.0 μg protein per dose (0.1 ml). It was provided courtesy of Dr. Rees, and shipped by air to Malawi, without thermal control.

The vaccine preparations were as follows:

BCG—Glaxo strain, freeze dried, batch G1693HA, given in a standard dose (0.03 mg moist weight = 1.6×10^6 viable particle count in 0.1 ml).

Killed *M. leprae*—whole *M. leprae* bacilli ("Lot 1") prepared at Wellcome Laboratories according to Draper protocol 1/79, under the auspices of IMMLEP ([13]). The bacilli were killed by exposure to 2.5 megarads gamma irradiation from a ^{60}Co source. This is the same lot of killed *M. leprae* as was used for vaccine in the Venezuelan ([3, 4]) and Norwegian ([9]) studies cited in Table 1. The vaccines were shipped to Malawi on dry ice at two dose concentrations: 2.5×10^7 bacilli per 0.1 ml and 7.5×10^7 bacilli per 0.1 ml.

BCG + killed *M. leprae*—the combined vaccines were made up in the field, by using killed *M. leprae* suspensions as above, to resuspend the freeze-dried BCG pellet.

This study was carried out within the context of the Lepra Evaluation Project (LEP), a total population longitudinal study in Karonga District, in northern Malawi (unpublished data). In the course of this survey, field teams consisting of paramedical workers and interviewers visited households systematically in order to interview and examine all members. The sensitization study represented only a slight departure from the routine work, in that skin testing was a regular part of the LEP.

The purpose of the study was explained to village leaders and to individuals in the study area to encourage their participation. Children less than 6 months of age, children of any age with malnutrition, individuals considered severely ill from any cause, leprosy cases and suspected leprosy cases were not included in the study. The study participants were recruited successively into seven groups, as illustrated in Table 2.

Initial skin testing and vaccination were carried out after interview and examination, on day 0. Soluble antigen skin tests were performed on the volar surface of the right forearm in all groups, and on both forearms in the non-vaccinated group 2. All soluble antigen skin tests involved intradermal injection of 0.1 ml of the reagent. Vaccine preparations were injected intradermally at the deltoid insertion area of the left arm, the total doses being divided between two sites approximately two inches apart. All injections were performed using standard tuberculin syringes (1 ml) fitted with 25-gauge needles.

Skin test reactions were read on the second or third day after injection. Induration diameters were read along and across the

TABLE 2. *Summary of protocol and numbers of subjects included in LEP sensitization study.*

Group no.	No. of persons	Day 0 Injections		Day 28	Day 90[a]	
		Left arm[b]	Right arm[c]	Vaccine sites examined	No. of persons	Proportion of intake
		Pilot Phase				
1	224	BCG + 5×10^7 KML	RT-23 tuberculin	350	—	
2A	127	RT-23 tuberculin	WEL-1	—	89	0.70
2B	123	RT-23 tuberculin	CD-19	—	91	0.74
		Vaccines—Phase I—Low Dose				
3A	56	BCG	WEL-1	110	45	0.80
3B	225	BCG	CD-19	342	148	0.66
4A	157	5×10^7 KML	WEL-1	252	101	0.64
4B	121	5×10^7 KML	CD-19	200	89	0.74
5A	156	BCG + 5×10^7 KML	WEL-1	164	49	0.31
5B	154	BCG + 5×10^7 KML	CD-19	206	68	0.44
		Vaccines—Phase II—High Dose				
6A	156	1.5×10^8 KML	WEL-1	244	95	0.61
6B	135	1.5×10^8 KML	CD-19	250	94	0.70
7A	215	BCG + 1.5×10^8 KML	WEL-1	262	118	0.55
7B	187	BCG + 1.5×10^8 KML	CD-19	296	108	0.58
Totals	2036			2676	1095	

[a] All groups, except group 1, received WEL-1 and CD-19 soluble antigens from purified *M. leprae* on opposite volar forearms, 0.1 ml intradermally. Group 1 received no skin test injections at day 90.

[b] BCG (0.03 mg) and killed *M. leprae* (KML) in the total dose indicated were given intradermally in 2 sites in the deltoid area. RT-23 tuberculin (groups 2A and 2B) was given intradermally in the volar forearm.

[c] RT-23 tuberculin (2 IU), soluble *M. leprae* antigen WEL-1 (0.5 μg), and soluble *M. leprae* antigen CD-19 (1.0 μg) were given intradermally in volumes of 0.1 ml in the right volar forearm.

arm and both diameters were recorded. The mean diameter of each site was used in the analyses.

Vaccination sites were examined as close as possible to four weeks after injection. In addition to the size of induration, the presence, size, and status (healed, healing, scabbed or still open) of any ulcers was recorded.

Follow-up skin tests with CD-19 and WEL-1 antigens were carried out three months after vaccination (day 90), on the volar surface of opposite forearms. These were read on the second or third day after injection.

It should be noted that group 1 (Table 2) represented a pilot study in order to assess the severity of ulcers or other reactions to a combined BCG plus low-dose killed-*M. leprae* vaccine in this population. It was agreed beforehand to stop the study if there were any serious adverse reactions to this vaccine, or if the procedure was unacceptable to the population. In addition, it was agreed beforehand not to proceed to groups 6 and 7 (high dose vaccines) if the incidence rate of ulcers equal to or greater than 10 mm at four weeks was greater than 5% in groups 4 or 5 (given low-dose vaccines). In neither case was it necessary to discontinue the study.

All injections and examinations took place at the homes of the participants. Limited "blindness" in reading skin tests was achieved by coding the antigens rather than by randomizing sites of injection.

RESULTS

A total of 2036 individuals were recruited into the study, and thus vaccinated and/or skin tested on day 0. Of the 1786 individuals given one or another vaccine preparation (i.e., all but the unvaccinated control group 2), 1331 (75%) were seen four weeks later and had their vaccination sites inspected. Of the 1812 individuals initially tested with a *M. leprae* soluble antigen (groups 2–7), 1095 (60%) were retested with both soluble antigens three months later.

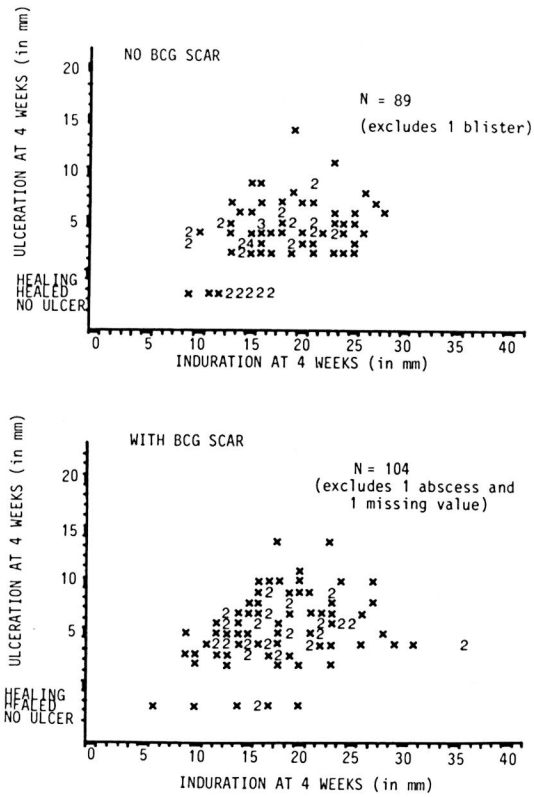


FIG. 1a. Relationship between ulceration and induration at vaccination sites 4 weeks after injection of 2.5×10^7 killed *M. leprae* plus 0.015 mg BCG into each of two sites (total dose = 5×10^7 killed *M. leprae* plus 0.03 mg BCG per person). Data are presented separately for persons with and without prior BCG scar. Single observations are represented by x's; numbers represent multiple observations of identical size combinations (group 5 from Table 2, individuals born since 1970).

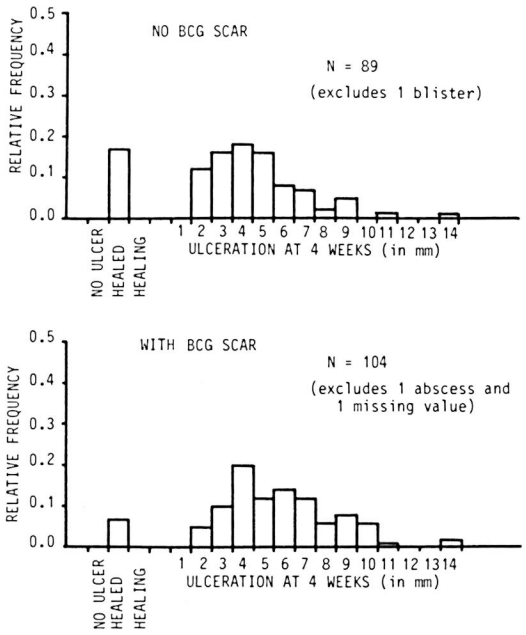


FIG. 1b. Relative frequency distributions of diameters of vaccination site ulcers 4 weeks after injection of 2.5×10^7 killed *M. leprae* plus 0.015 mg BCG into each of two sites (total dose = 5×10^7 killed *M. leprae* plus 0.03 mg BCG per person) (same individuals as shown in Fig. 1a).

Detailed results for individuals who received 5×10^7 killed *M. leprae* + 0.03 mg BCG are shown in Figures 1a–1d. The results for each of the groups are summarized in Table 3, broken down by age (greater or less than 14 years) and BCG status (as assessed by the presence or absence of a BCG-like scar on the right deltoid).

Vaccine acceptability. Ulceration results are presented in Table 3 per vaccination site (two per person). Preparations incorporating BCG were more likely to cause ulceration than were those containing killed *M. leprae* alone. Ulcers occurred at 100% of the sites receiving the combined killed *M. leprae* plus BCG preparations.

The size ranges of vaccination site ulcers are presented as observed four weeks after vaccination. It should be noted that although the protocol called for the examination of the vaccination sites at four weeks, it became evident in the field that the reactions were generally largest at two to three weeks after injection. This must be taken into consideration when assessing the results. It is seen that ulcer sizes varied with three factors: a) they were larger in persons with, than in those without, BCG scars; b) they were larger subsequent to vaccination with BCG-containing preparations than with killed *M. leprae* alone; and c) they were correlated with the dose of killed *M. leprae,* being largest subsequent to vaccination with 0.03 mg BCG combined with the higher dose (1.5×10^8) of killed *M. leprae* (half this dose having been injected into each site).

The relationship between prior tuberculin sensitivity and the size of ulcers caused by the 5×10^7 killed *M. leprae* plus BCG vaccine is shown in Figure 2. There was a correlation between these measures, in particular among individuals less than 14 years of age ($r = 0.4$, $p < 0.05$).

TABLE 3. *Results of LEP 1984 sensitization study. The study population separated by age (born before or after 1 Jan. 1970) and by BCG scar status.*[a]

	Born ≥1970			Born <1970			Grand totals
	BCG−	BCG+	Total	BCG−	BCG+	Total	
Pilot group 1 [=5 × 10^7 killed *M. leprae* + 0.03 mg (=1.6 × 10^6) viable BCG]							
Ulcerations	54/54 (1.00)	120/120 (1.00)	174/174 (1.00)	128/128 (1.00)	48/48 (1.00)	176/176 (1.00)	350/350 (1.00)
Ulcer size (mm)	2–14	1–12	1–14	2–25[b]	2–15	2–25	1–25
Control group 2 (No vaccine)							
WEL-1 conv.							4/85 (0.05)
WEL-1 (90) range							3–10
CD-19 conv.							21/54 (0.39)
CD-19 (90) range							8–21
Group 3 [=0.03 mg (=1.6 × 10^6) viable BCG]							
Ulcerations	94/112 (0.84)	90/92 (0.98)	184/204 (0.90)	161/166 (0.97)	82/82 (1.00)	243/248 (0.98)	427/452 (0.94)
Ulcer size (mm)	1–5	1–9	1–9	1–9	2–12	1–12	1–12
WEL-1 conv.	3/12 (0.25)	8/12 (0.67)	11/24 (0.46)	5/12 (0.42)	3/9 (0.33)	8/21 (0.38)	19/45 (0.42)
WEL-1 (90) range	2–13	2–17	2–17	2–20	2–15	2–20	2–20
CD-19 conv.	23/27 (0.85)	16/17 (0.94)	39/44 (0.89)	29/32 (0.91)	10/11 (0.91)	39/43 (0.91)	78/87 (0.90)
CD-19 (90) range	7–20	7–26	7–26	4–22	2–30	2–30	2–30
Group 4 [=5 × 10^7 killed *M. leprae*]							
Ulcerations	62/95[c] (0.65)	85/111[c] (0.77)	147/206 (0.71)	116/179[c] (0.65)	55/67[c] (0.82)	171/246 (0.70)	318/452 (0.70)
Ulcer size (mm)	2–4	1–9	1–9	1–6	2–6	1–6	1–9
WEL-1 conv.	12/23 (0.52)	10/22 (0.45)	22/45 (0.49)	10/37 (0.27)	10/19 (0.53)	20/56 (0.36)	42/101 (0.42)
WEL-1 (90) range	1–15	3–16	1–16	2–17	2–16	2–17	1–17
CD-19 conv.	5/7 (0.71)	12/14 (0.86)	17/21 (0.81)	7/12 (0.58)	3/5 (0.60)	10/17 (0.59)	27/38 (0.71)
CD-19 (90) range	4–23	5–24	4–24	7–23	6–20	6–23	4–24

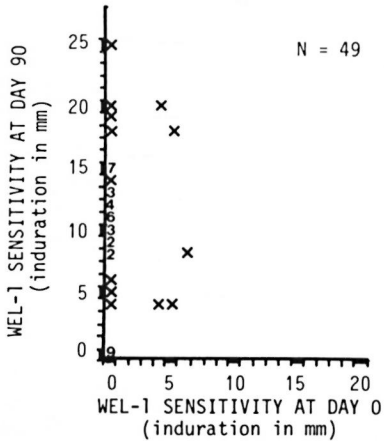
TABLE 3. CONTINUED.

	Born ≥1970			Born <1970			Grand totals
	BCG−	BCG+	Total	BCG−	BCG+	Total	
Group 5 [=5 × 10^7 killed *M. leprae* + 0.03 mg (=1.6 × 10^6) viable BCG]							
Ulcerations	89/89[c] (1.00)	105/105[c] (1.00)	194/194 (1.00)	120/120 (1.00)	54/54 (1.00)	174/174 (1.00)	368/368 (1.00)
Ulcer size (mm)	2–14	2–14	2–14	2–11	2–16	2–16	2–16
WEL-1 conv.	12/15 (0.80)	14/19 (0.74)	26/34 (0.76)	6/10 (0.60)	3/4 (0.75)	9/14 (0.64)	35/48 (0.73)
WEL-1 (90) range	4–23	3–25	3–25	6–19	6–15	6–19	3–25
CD-19 conv.	6/7 (0.86)	11/11 (1.00)	17/18 (0.94)	7/9 (0.78)	4/4 (1.00)	11/13 (0.85)	28/31 (0.90)
CD-19 (90) range	12–31	8–37	8–37	5–27	15–27	5–27	5–37
Group 6 [=1.5 × 10^8 killed *M. leprae*]							
Ulcerations	80/104 (0.77)	133/138 (0.96)	213/242 (0.88)	158/178 (0.89)	70/74 (0.95)	228/252 (0.90)	441/494 (0.89)
Ulcer size (mm)	1–9	1–11	1–11	1–9	1–7	1–9	1–11
WEL-1 conv.	11/24 (0.46)	21/25 (0.84)	32/49 (0.65)	21/36 (0.58)	6/8 (0.75)	27/44 (0.61)	59/93 (0.63)
WEL-1 (90) range	4–23	3–21	3–23	4–19	3–23	3–23	3–23
CD-19 conv.	15/16 (0.94)	27/27 (1.00)	42/43 (0.98)	15/16 (0.94)	5/5 (1.00)	20/21 (0.95)	62/64 (0.97)
CD-19 (90) range	10–28	9–29	9–29	9–28	5–25	5–28	5–29
Group 7 [=1.5 × 10^8 killed *M. leprae* + 0.03 mg (=1.6 × 10^6) viable BCG]							
Ulcerations	133/133[c] (1.00)	173/173[c] (1.00)	306/306 (1.00)	160/160 (1.00)	92/92 (1.00)	252/252 (1.00)	558/558 (1.00)
Ulcer size (mm)	1–12	1–13	1–13	1–15	2–30[d]	1–30	1–30
WEL-1 conv.	20/21 (0.95)	35/40 (0.88)	55/61 (0.90)	20/26 (0.77)	21/23 (0.91)	41/49 (0.84)	96/110 (0.87)
WEL-1 (90) range	3–18	4–19	3–19	2–20	5–26	2–26	2–26
CD-19 conv.	21/21 (1.00)	11/11 (1.00)	32/32 (1.00)	13/14 (0.91)	6/6 (1.00)	19/20 (0.95)	51/52 (0.98)
CD-19 (90) range	10–25	11–32	10–32	5–26	14–29	5–29	5–32

[a] For each vaccine preparation, results are expressed per vaccination site (2 per individual) as:

Ulcerations = Proportion with evidence of ulceration at vaccine site at 4 weeks.
Ulcer size (mm) = Range of vaccine site ulcer sizes at 4 weeks.
WEL-1 conv. = WEL-1 conversion rate, defined as proportion of those initially ≤5 mm who were >5 mm 90 days after vaccination.
WEL-1 (90) range = Range of indurations to WEL-1 at 90 days in mm, excluding those which were 0.
CD-19 conv. = CD-19 conversion rate, defined as above.
CD-19 (90) range = Range of indurations to CD-19 at 90 days in mm, excluding those which were 0.

[b] The 25 mm ulcer was an outlier; second largest ulcer in this group was 14 mm.
[c] Odd number due to omission of abscess, blister, or missing observation.
[d] The 30 mm ulcer was an outlier; second largest ulcer in this group was 19 mm.



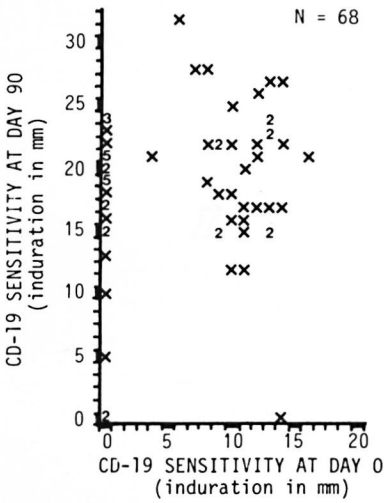
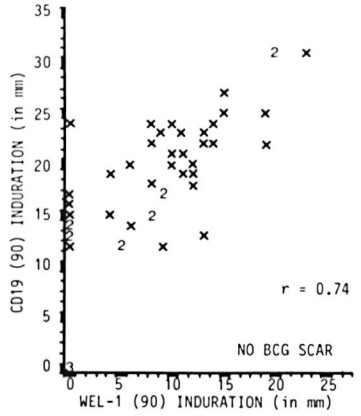


FIG. 1c. Scatter diagrams showing relationship between skin test sensitivity to WEL-1 and CD-19 antigens before ("day 0") and 90 days after vaccination with total of 5×10^7 killed *M. leprae* plus 0.03 mg BCG. Single observations are represented by x's; numbers represent multiple observations of identical size combinations (group 5 from Table 2).



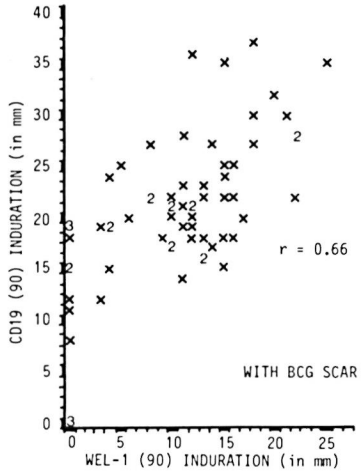


FIG. 1d. Scatter diagrams showing relationship between sensitivity to CD-19 and WEL-1 skin tests, 90 days after vaccination with total of 5×10^7 killed *M. leprae* plus 0.03 mg BCG. Data are presented separately for persons with and without prior BCG scar. Single observations are represented by x's; numbers represent multiple observations of identical size combinations (group 5 from Table 2).

Given that BCG itself causes ulcers but is considered acceptable as a vaccine, it is of interest to use it as a reference when assessing vaccine site reactions. Figure 3 illustrates the cumulative frequency distributions of ulcers observed subsequent to vaccination with BCG and with the combination of BCG and low-dose (5×10^7) killed *M. leprae*.

The proportion of individuals who refused or otherwise avoided a follow-up skin test at three months may be taken as an indicator of the discomfort and dissatisfaction with the different vaccines. The relationship is not simple, insofar as there was inevitably some variation in the effort and persuasiveness of different field workers and in the level of cooperation between different villages. Nonetheless, it is of interest that the 90-day follow-up rates were lowest for the two groups (5 and 7) which received combined killed *M. leprae* plus BCG vaccines.

Sensitizing effects. Most individuals received only one *M. leprae* soluble antigen prior to vaccination, but were tested with

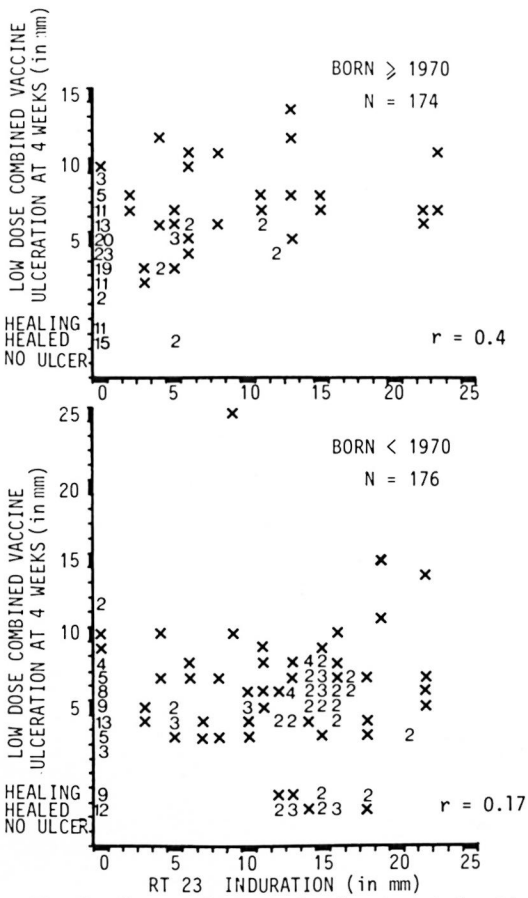


FIG. 2. Scatter diagrams showing the relationship between prior tuberculin (RT-23) sensitivity and the diameters of vaccination site ulcers 4 weeks after injection of 2.5×10^7 killed *M. leprae* plus 0.015 mg BCG into each of two sites (total dose = 5×10^7 killed *M. leprae* plus 0.03 mg BCG per person). Data are presented separately for individuals born before or after 1 Jan. 1970. Single observations are represented by x's; numbers represent multiple observations of indentical size combinations (group 1 from Table 2).

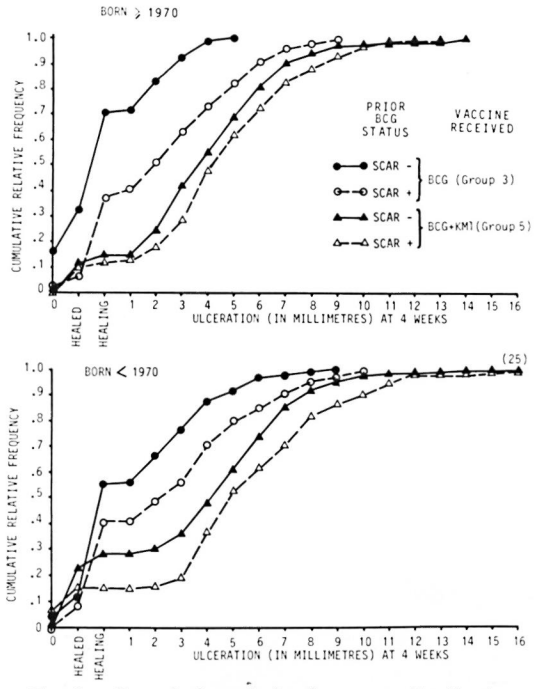


FIG. 3. Cumulative relative frequency distributions of ulcer diameters 4 weeks after injection of 0.015 mg BCG or 2.5×10^7 killed *M. leprae* plus 0.015 mg BCG into each of two sites (total dose = either 0.03 mg BCG or 5×10^7 killed *M. leprae* plus 0.03 mg BCG per person). Data are shown separately for persons born before or after 1 Jan. 1970, and according to BCG scar status at time of vaccination (groups 3 and 5 from Table 2).

both the WEL-1 and CD-19 antigens at 90 days. Examples of the frequency distributions of skin test indurations at day 0 and day 90 are illustrated in Figures 1c, 1d, and 4. It is important to note that the vast majority of the WEL-1 tests showed no induration at all prior to vaccination in all groups.

A simple method to measure the sensitizing effect is by examining the proportions converting to "positive" (taken arbitrarily as an induration of greater than 5 mm) at 90 days after vaccination among those who were initially "negative" (less than or equal to 5 mm) at the time of vaccination. These figures are given in Table 3. In order to distinguish the vaccine-attributable effects from changes due either to the initial skin test itself or to changes in technique or reading, it is necessary to compare changes in skin test sensitivity observed in the vaccinated groups with those observed in the non-vaccinated controls (group 2). These results are shown in Figure 4. Of 85 individuals initially considered "negative" (less than 6 mm) to WEL-1, 4 (5%) were recorded as "positive" when retested 90 days later. In contrast, of the 54 individuals initially "negative" to the CD-19 antigen, 21 (39%) were considered positive 90 days later. An inspection of Figure 4 reveals an appreciable degree of natural "reversion" in these tests as well—i.e., 3 out of 4 initial "positives" to WEL-1 were "negative" when retested and 11 of 37 (30%) of initial "positives" to CD-19 were negative when retested after three months.

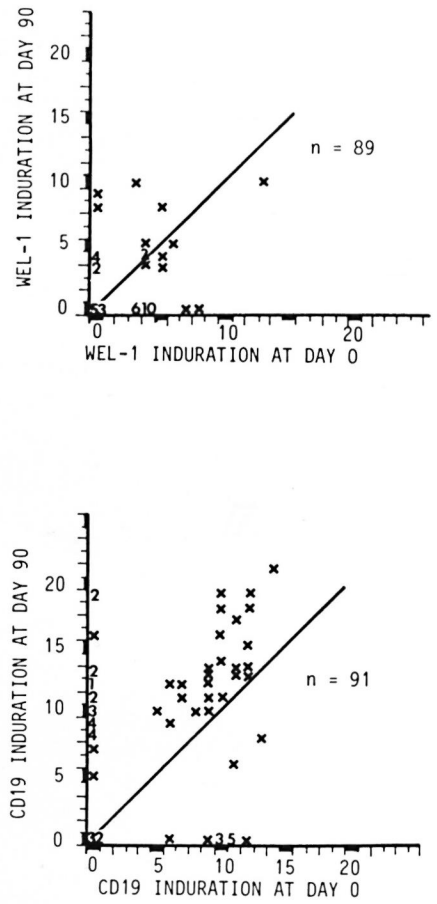


FIG. 4. Scatter diagrams showing changes in WEL-1 and CD-19 skin test sensitivity over 3 months in unvaccinated individuals. Single observations are represented by x's; numbers represent multiple observations of identical size combinations (group 2 from Table 2).

The apparent "conversion rates" were consistently higher when measured in terms of CD-19 than in terms of the WEL-1 skin test antigens.

Overall conversion rates to the WEL-1 antigen were lowest after BCG alone or after low-dose killed *M. leprae* (42% for both), higher among those receiving the high-dose killed *M. leprae* alone (63%), and highest among those who received the combined BCG plus killed *M. leprae* preparations (73% for low dose and 87% for the high dose).

On the other hand, the CD-19 overall conversion rates were lower in the group receiving low-dose killed *M. leprae* alone (71%) than in those receiving BCG alone (90%). This difference is significant ($\chi^2 = 5.49$, $p < 0.05$). Similar conversion rates were observed among individuals receiving BCG plus low-dose killed *M. leprae* vaccine (also 90%). Very high conversion rates were observed in the groups receiving the higher dose of killed *M. leprae*, either without (97%) or with (98%) BCG.

DISCUSSION

This study should be viewed in the context of current knowledge of skin test sensitization to and by antigens of *M. leprae*. In addition to the vaccine studies summarized in Table 1, in which sensitization has been assessed in terms of *M. leprae* soluble antigen skin tests, a number of studies have shown that either BCG or Mitsuda lepromin (in effect heat-killed *M. leprae*, conventionally given intradermally at a dose of 1.6×10^7 organisms) can induce sensitivity as assessed by the delayed (four week) "Mitsuda" reaction to lepromin itself ([1, 5, 6]). These lepromin "conversion" studies have been difficult to interpret: first, because the sensitization assay (Mitsuda lepromin) is itself a sensitizer and appropriate controls are generally lacking and, second, because the immunological mechanism and implications of the four-week Mitsuda response are not understood. Insofar as the mechanism underlying the 48–72 hour skin test response is reasonably well understood, the use of such tests with *M. leprae* soluble antigens may appear a more reasonable method for screening the immunogenicity of leprosy vaccines.

The study reported here provides information on the acceptability and the sensitizing potential of five different vaccines in a leprosy-endemic population in Africa.

Ulceration occurred in a large proportion of the recipients of all vaccines, and in 100% of those who received combined *M. leprae* plus BCG vaccines. Ulceration itself may not be considered an unacceptable side effect of a vaccine, given that most populations have accepted both BCG and smallpox vaccinations. On the other hand, this side effect may be deemed acceptable only if there is a high risk of leprosy in a community, and if there is a reasonable chance that the vaccine will be protective.

In interpreting the results given in this paper, it must be emphasized that the vaccine-site lesions were examined four weeks after vaccination, when they were past their

peak severity in terms of ulcer size. In addition, it should be borne in mind that all of the vaccines used in this study were divided between two sites on the arm, each site receiving half the stated dose. Nonetheless, we may use the BCG group 3 results for a standard and a precedent when assessing the severity of these reactions. Our data suggest that neither 5×10^7 nor 1.5×10^8 killed *M. leprae* given alone caused ulceration which was as severe or more severe than the standard dose (0.03 mg) BCG. There was an increase in both the frequency and size of ulcers among individuals who received the combined low-dose killed *M. leprae* plus BCG combination. The high-dose combined vaccine (group 7) caused ulcers which were yet larger and, in some individuals, caused metastatic indurations and ulcers. This dosage was, in fact, considered unacceptable by some of the field staff involved.

Although the subjects were not questioned formally as to their attitudes toward the vaccine reactions, the proportion of individuals who accepted the follow-up skin test 90 days after vaccination was lowest among the two groups who received the combined *M. leprae* plus BCG vaccines. This is probably an indication of some dissatisfaction on their part.

Our findings on the relationship between prior tuberculin reaction and ulceration following vaccination with the combined low-dose killed *M. leprae* plus BCG vaccine are of interest. In order to minimize severe side reactions, the ongoing vaccine trial in Venezuela recommends 40% of the standard dose of BCG among individuals who are strongly tuberculin positive and a double dose of BCG among those with tuberculin reactions of less than 10 mm ([3]). Our results show there is some relationship between vaccine site ulceration and prior tuberculin status (Fig. 2), but we question whether the resulting small reduction in ulcer size would warrant the trouble of adjusting the dose on this account, at least in this African population.

With respect to the sensitizing potential of the several vaccines, we have used an arbitrary measure of >5 mm as a "positive," and have presented conversion rates as the percent of those who were initially 0–5 mm who were >5 mm when retested three months after vaccination. It is important to note this criterion since different results between studies (e.g., Table 1) may be due in part to different conventions in reading and in interpreting skin test reactions.

Our results indicate higher "conversion" rates using the CD-19 than the WEL-1 antigen. What is more, the specificity of the sensitization appeared to differ between the two antigens, insofar as the "conversion" rates subsequent to BCG alone were only 42% for the WEL-1 antigen as compared to 90% for the CD-19. WEL-1 "conversion" appeared considerably higher after combined killed *M. leprae* plus BCG vaccines than after either BCG or killed *M. leprae* alone. Very high rates of CD-19 conversion were noted after high doses of killed *M. leprae* either with or without BCG.

These results are difficult to interpret. The antigenic compositions of the WEL-1 and CD-19 antigens are not known. Not only were these antigens produced by different protocols, but we know that there is considerable variation in the behavior of different batches of antigen prepared by either protocol ([8]). In this context, it should be noted that the CD-19 batch of NIMR-produced antigen used in this investigation was identical to that employed in the Norway study cited in Table 1 ([9]). Different batches of NIMR antigen were used in the Malaysia ([12]). and Nepal ([11]) studies. The WEL-1 batch employed in this investigation has also been used in Venezuela, where it was shown to be a less-sensitive indicator than was a locally produced ("IND") batch of antigen produced by a similar protocol (Table 1, ref. 4). There was an unexpected degree (39%) of "conversion" to the CD-19 antigen observed in the unvaccinated control group in our studies. It is not known to what extent this may reflect temporal variations in skin sensitivity to this antigen among members of this population or boosting of the skin test response or actual sensitization by the initial skin test. Perhaps both of these explanations are involved. The difference between natural conversion rates to the WEL-1 and CD-19 antigens is unlikely to be due to inter-observer variation since the indurations in both groups (2A and 2B) were read by the same staff. If we assume a 39% background conversion among prior nega-

tives in the control group, then the "corrected" CD-19 conversion rates would be 52% subsequent to 5×10^7 killed *M. leprae*, 84% subsequent to BCG or to combined BCG + 5×10^7 killed *M. leprae*, and 95% and 97% subsequent to 1.5×10^8 killed *M. leprae* without and with BCG, respectively.

Finally, we must emphasize that although these results indicate that several of the vaccine preparations discussed here might be acceptable at least insofar as they cause ulcerations no worse than does BCG, and that they may sensitize a high proportion of recipients, we are a long way from understanding the vaccine potential of the preparations. Skin test sensitivity is not identical with protection against disease. Indeed, studies in tuberculosis indicate that the two may not even be correlated ([10]). But protection can only be demonstrated in field trials, and studies such as those reported here are necessary if we are to make wise choices in planning and interpreting such trials in the future.

SUMMARY

This paper describes a comparison between BCG alone and two different doses of killed *Mycobacterium leprae*, with or without BCG, in stimulating skin-test sensitivity to two different soluble antigens prepared from *M. leprae*. Skin test conversion was assessed three months after vaccination. Significant rates of skin test conversion were stimulated by each of the vaccines to both skin test antigens, but the observed conversion rates differed markedly as measured by the two antigens. All of the vaccines caused ulcers at the site of injection in most subjects, and these local reactions are described. A combined vaccine containing 0.03 mg BCG plus 5×10^7 killed *M. leprae* induced high rates of "conversion" to both skin tests but caused local reactions slightly larger than those caused by BCG alone. The implications of these findings for selection of an optimal vaccine formulation for use in large-scale preventive trials are discussed.

RESUMEN

Este trabajo compara la capacidad del BCG solo con la de dos diferentes dosis de *Mycobacterium leprae* muerto, con o sin BCG, para estimular la reactividad en piel hacia 2 antígenos solubles diferentes preparados a partir del *M. leprae*. La reactividad en piel se valoró 3 meses después de la vacunación. Ambas vacunas estimularon la positividad de las pruebas dérmicas hacia los 2 antígenos de prueba pero los grados de conversión difirieron marcadamente. Todas las vacunas causaron úlceras en el sitio de inyección en al mayoría de los sujetos. Estas reacciones locales se describen en el trabajo. Una vacuna combinada conteniendo 0.03 mg de BCG más 5×10^7 *M. leprae* muerto, indujo altas frecuencias de conversión hacia los 2 antígenos de prueba pero causó reacciones locales ligeramente mayores que las causadas por el BCG solo. Se discuten las implicaciones de estos hallazgos en cuanto a la selección de preparados vacunales propuestos para su uso en programas preventivos a gran escala.

RÉSUMÉ

Ce travail compare les effets de stimulation de la sensibilité de l'épreuve cutanée à deux antigènes solubles différents préparés à partir de *Mycobacterium leprae*, suite à l'utilisation de BCG seul et de deux doses différentes de *M. leprae*, avec ou sans BCG. Les épreuves de virage de l'épreuve cutanée ont été évaluées trois mois après la vaccination. Chacun de ces vaccins a stimulé, dans une mesure significative, le virage de l'épreuve cutanée pour les deux antigènes cutanés étudiés; les taux de virage qui ont été observés différaient cependant de manière notable entre l'un et l'autre antigènes. Chacun de ces vaccins a causé des ulcères à l'endroit de l'infection, et ceci chez la plupart des sujets; ces réactions locales sont décrites. Un vaccin combiné contenant 0,03 mg de BCG, additionné de 5×10^7 *M. leprae* tués, a entraîné un taux élevé de virage des épreuves cutanées, mais a entraîné cependant des réactions légèrement plus étendues que celles produites par le BCG seul. On discute des conséquences de ces observations, en vue du choix d'un régime optimal de vaccination des essais préventifs sur une large échelle.

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