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Effects of ICRC Antileprosy Vaccine in Healthy Subjects[1]

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A vaccine containing ICRC bacilli, a group of cultivable mycobacteria belonging to the *Mycobacterium avium intracellulare* complex, has been in use for the last 7 years. During this period, the vaccine has been administered to more than 100 leprosy patients and a number of lepromin-negative healthy subjects who represent a high-risk group. The vaccine brought about lepromin conversion in 55% of lepromatous leprosy (LL) patients and 95% of healthy subjects, the conversion being associated with upgrading of the lesions in the patient ([1, 10, 11]). Since the lepromin reaction correlates well with host immunity against leprosy ([12, 17, 21, 23]), the duration of conversion in lepromin-negative vaccinated subjects would be a good index of the stability of vaccine-induced immunity.

The vaccine was cleared 2 years ago by the Drug Controller of India for phase-III clinical trials to assess its immunoprophylactic efficacy. Until then, the vaccine had been tried in LL patients, their lepromin-negative, healthy household contacts, and noncontacts in the general population. For reasons discussed elsewhere ([9]), in the proposed phase-III trials the vaccine will be administered to healthy volunteers irrespective of their lepromin status.

Hypersensitivity to *M. leprae* antigens has been implicated in the pathogenesis of nerve damage in leprosy ([8, 16, 18]) to which residents of endemic areas are exposed continuously. The vaccine could induce nerve damage, especially in lepromin-positive subjects. Before embarking on large-scale phase-III trials, it was, therefore, essential to conduct studies to establish that the vaccine did not produce any untoward reactions in lepromin-positive subjects. During the last 2 years, this facet was investigated in both lepromin-positive and lepromin-negative household contacts of leprosy patients. In some of the vaccinated subjects, circulating antibodies against *M. leprae*-specific phenolic glycolipid I (Ab-PGL) were also quantitated before and after vaccination. In this communication, we report our experience with vaccination of healthy subjects.

MATERIALS AND METHODS

Vaccination of lepromin-negative subjects

This part of the study was conducted in healthy volunteers of both sexes between 5–55 years of age in Malwani, a suburb of Bombay with a population of about 63,000. Seth G. S. Medical College, a leading medical institution in Bombay, has established a primary community health center in Malwani which was used as the base. The residents of Malwani belong to the economically lower middle or poor class. There are a total of 691 leprosy patients, giving a prevalence rate of 10.6/1000. There is no segregation of patients. The vaccine was given to two groups of healthy lepromin-negative subjects of both sexes consisting of a) household contacts of multibacillary (lepromatous–borderline lepromatous or LL-BL) patients and b) noncontacts in the general population.

Household contacts. One hundred thirty-four household contacts from 35 families were subjected to the lepromin test using Mitsuda antigen. Each family had at least one index case of LL-BL. Although the patients had been on drugs for periods varying

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from 2 to 5 years, all were index cases at the time of the initiation of the study. They had received mostly dapsone. In some, rifampin was also administered. Each contact was thoroughly examined for evidence of leprosy, and only those who were clinically free of the disease were accepted for vaccination. Of the 37 contacts who were lepromin negative, 26 volunteered for the study. Nineteen received the vaccine and the other 7, who only received saline, served as controls. A lepromin test was performed before vaccination and repeated at 8 weeks, and 1 and 3 years after vaccination. A special note was made of the BCG scar. Each volunteer received, intradermally in the deltoid region, a single injection of 0.1 ml of the vaccine containing 0.5×10^9 ICRC bacilli killed by gamma-irradiation. Each control likewise received 0.1 ml of saline.

Noncontacts. Studies on the general population were carried out on a school-age group. Four hundred school children of both sexes, between 10–18 years of age, were simultaneously subjected to lepromin and tuberculin tests. A special note was made of the BCG scar. Of the 56 children who were lepromin negative, 20 volunteered for the study. They received the vaccine in a dose of 1.7×10^7 ICRC bacilli/person. The purpose of using this dose was to find out the lower concentration of the organisms at which the vaccine could be effective. Twenty-four children who received only saline served as controls. A lepromin test was performed before vaccination, and 8 weeks and 1 year after vaccination.

Vaccination in healthy household contacts irrespective of their pre-vaccination lepromin reactivity

This part of the study was conducted in the townships of Satpati and Palghar. The former is a costal town about 100 km north of Bombay. Palghar is about 7 km southeast of Satpati. The total population of the two towns is about 35,000, and the total number of leprosy patients is 620, giving a prevalence rate of 18/1000. The multibacillary type of leprosy constitutes about 20% of the cases. A lepromin test was performed using Mitsuda antigen on 373 healthy household contacts of 70 leprosy patients. The patients, who had received dapsone for varying periods, showed variable bacteriological status. The vaccine was administered in a dose of 0.5×10^9 ICRC bacilli/person to 158 contacts, including 69 females, between 5–60 years of age, irrespective of their lepromin status. The lepromin reaction was negative in 29 of the vaccinated volunteers. No sex differences were observed in lepromin reactivity. The test was repeated in 38 and 19 mutually exclusive subjects at 8 and 20 months after vaccination. This was done to eliminate the effects, if any, of repeated lepromin testing on lepromin reactivity. Finger-prick blood samples were also collected from some subjects before and after vaccination for quantitation of circulating Ab-PGL.

Skin tests

Lepromin (Mitsuda) test. The Mitsuda test was performed using 0.1 ml of integral lepromin containing 4×10^7 bacilli/ml. The antigen was given on the volar surface of the left forearm. Lepromin was obtained through the kind courtesy of Dr. W. F. Kirchheimer, GWL Hansen's Disease Center, Carville, Louisiana, U.S.A., with the assistance of the World Health Organization (WHO). The local response was recorded at 3 weeks. An induration of 3 mm and above denoted a positive response.

Tuberculin reaction. PPD, obtained from the BCG Laboratory, Madras, India, was used to perform the test. The antigen was given intradermally on the volar surface of the right forearm in the dose of 2 IU/person. Erythema/induration of >10 mm at 72 hr denoted a positive response.

Enzyme-linked immunosorbent assay (ELISA)

An ELISA was used to measure circulating Ab-PGL using essentially the method described by Young, *et al.* ([28]). An aliquot of 20 μl of blood from a finger prick was dispensed from a capillary tube (Micropet), onto a filter paper disc (Whatman No. 1, 2 mm in diameter), allowed to dry, and stored at -20°C until used. The serum was eluted from filter discs in 2 ml of phosphate buffered saline (PBS) with 5% goat serum for 1 hr at room temperature. ELISA plates, 96-well PVC (Costar, Cambridge, Massachusetts, U.S.A.), were coated with 0.1 ml of 5 μg/ml of PGL-I for 1 hr at 37°C. The plates were then washed four times with PBS and

TABLE 1. *Lepromin reactivity in household contacts of leprosy patients.*[a]

Age group	Sex	No.	Lepromin reaction (mm)							
			<3 (Negative)		3–5		5–7		>7 or ulceration	
			No.	%	No.	%	No.	%	No.	%
Children 5–10 yrs	Male	20	11	55.0	9	45.0	—		—	
	Female	17	8	47.06	6	35.29	3	17.65	—	
Adolescents and young adults 10–20 yrs	Male[b]	23	5	21.74	13	56.52	4	17.39	1	4.35
	Female[c]	27	6	22.22	14	51.86	7	25.92	—	
Adults 20 or more yrs	Male[b]	16	2	12.5	7	43.75	3	18.75	4	25.0
	Female[c]	31	5	16.13	13	41.93	8	25.81	5	16.13

[a] Chi-square test with Yates correlation has been applied to test the differences between lepromin reaction <3 and >3 mm between adolescent and adult age groups.
[b] Male: $\chi^2 = 0.09947$, d.f. = 1, $p > 0.5$, not significant.
[c] Female: $\chi^2 = 0.06487$, d.f. = 1, $p > 0.5$, not significant.

incubated with 5% bovine serum albumin (BSA) in PBS at 37°C for 2 hr (0.1 ml per well). The BSA was aspirated and replaced with 0.1 ml of test sera (from filter discs). Incubation was carried out for 2 hr at 37°C, the plates were washed four times with PBS, and 0.1 ml of 1:1000 dilution of goat anti-human globulin (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) was added per well. The plates were incubated for 1 hr at 37°C, washed four times with PBS, and 0.1 ml of substrate (0.1 mg/ml *o*-phenylenediamine in 0.01 M citrate buffer, pH 5.0, with 0.003% hydrogen peroxide) was added to each well. The plates were incubated in the dark at room temperature for 20 min. The reaction was stopped by the addition of 8 N H_2SO_4 and color development was measured at 492 nm using an automatic ELISA reader. All of the samples, including controls, were run in duplicate. The antibody level was calculated by subtracting the mean optical density (OD) of the negative controls from the mean OD of the test samples. The results were expressed as the fraction of the mean value of the pooled LL sera which were collected from 10 untreated LL patients with high bacterial index; 2 ml of serum from each patient was pooled.

Vaccine

The vaccine was prepared from ICRC strain C-44 that had been isolated in 1969 from a leproma. The organism, which was in the 68th passage at the time of vaccination, was killed by gamma-irradiation using 500 K rads. Details of the vaccine preparation are described elsewhere ([11]).

RESULTS

General observations

Volunteers were very closely monitored for clinical evidence of leprosy and any untoward side effects of the vaccination throughout the study. The administration of the vaccine did not produce any acute local reaction. However, 5–6 days after vaccination a low-grade inflammatory swelling, which ulcerated by the 15th day, was observed at the vaccination site in all subjects. Regional lymph node enlargement was seen in many household contacts who had received the high dose (0.5×10^9 bacilli/person) and also in a few noncontacts who were administered the low dose (1.7×10^7 bacilli/person). Lymph node enlargement was generally mild, lasted for about a week, and required no special treatment. No systemic reactions were observed. The ulcer had regular smooth margins and was about 1 cm in diameter. The underlying granulation tissue was healthy looking. If the ulcer was kept clean, generally no treatment was required. In some cases sulpha ointment 4% (IP) in paraffin base was applied for a few days. The ulcer was treated with local application and healed in about 2 weeks. The scar was similar to that seen after BCG vaccination. Thus, the vaccine was well tolerated. During the 3-year period of observa-

TABLE 2. *Vaccine-induced lepromin conversion in household contacts.*[a]

Sr. no.	Age/sex	BCG	PPD (mm)	Mitsuda reaction (mm)			
				Pre-vaccination	Post-vaccination		
					8 wks	1 yr	3 yrs
1	15/F	+	12	1	6 u[b]	6	5
2	10/M	+	10	0	5	7	4
3	11/M	+	3	0	6 u	8 u	6 u
4	40/F	−	0	0	7 u	6 u	8 u
5	25/F	+	15	1	5	6	ND[c]
6	10/F	+	12	0	5	6	ND
7	45/F	−	5	0	6	10 u	5
8	10/F	+	22	0	5	6	8 u
9	16/M	−	0	0	5	6	4 u
10	17/F	+	20	1	5 u	6 u	5 u
11	25/F	−	0	2	5 u	6	6
12	28/F	−	5	1	7 u	ND	5 u
13	46/M	−	10	1	7 u	ND	ND
14	46/F	−	5	0	5 u	ND	5
15	10/F	−	0	0	0	4	6
16	17/F	+	20	1	1	6 u	ND
17	7/F	−	5	0	0	0	0
18	8/M	−	5	0	4	5	ND
19	6/M	+	5	0	2	6	5
Total conversions (>3 mm)					15/19 (79%)	15/16 (94%)	13/14 (93%)

[a] In three controls, who were lepromin negative, the test was repeated at the intervals of 8 weeks, 1 year, and 3 years. None exhibited lepromin conversion.
[b] u = ulcer.
[c] ND = not done.

tion, the vaccinated volunteers remained healthy and none developed any evidence of leprosy. No sex-dependent differences were observed. The data are, therefore, pooled and discussed together.

Studies in lepromin-negative subjects

Household contacts. The results of the lepromin test conducted in the household contacts of the patients are shown in Table 1. As expected, lepromin reactivity improved with age—between 5–10 years, about 50% of children were negative; this figure dropped to about 15% in adults. Eight weeks after vaccination, 15 out of 19 (79%) individuals exhibited lepromin conversion (Table 2). At the end of 1 year, the test, which was repeated in 16 subjects, was positive in 15 (94%) of these. Thereafter, lepromin conversion and its intensity remained stable up to 3 years until the end of the study (Table 2).

Noncontacts. Table 3 presents a comparison of the lepromin reactivity in noncontacts in the age group 10–18 years and household contacts of similar ages. No differences were seen in the two groups. Table 4 shows that there was no correlation between lepromin reactivity, administration of BCG, and PPD reaction. At 8 weeks postvaccination, only 9 of 20 (45%) vaccinated subjects developed a positive response (Table 5). At the end of 1 year, 94% of the vaccinated children (15 out of 16) exhibited lepromin conversion; furthermore the average induration was larger in size as compared to that observed at 8 weeks. No correlation was observed between lepromin conversion and BCG vaccination or the status of tuberculin reaction.

Nonvaccinated subjects. Table 6 shows lepromin reactivity in 24 nonvaccinated, lepromin-negative household contacts and noncontacts. No conversions were observed as a consequence of the lepromin test.

Vaccination in volunteers irrespective of their pre-vaccination lepromin reaction

As shown in Table 7, all lepromin-negative individuals exhibited a strong reaction

TABLE 3. *Comparison of lepromin reactivity in household contacts and noncontacts in leprosy-endemic area.*[a]

Sex		Total	Lepromin (Mitsuda) reaction in mm							
			<3		3–5		5–7		7 ulceration	
			No.	%	No.	%	No.	%	No.	%
Males	Contacts	23	5	21.74	13	56.52	4	17.39	1	4.35
	Noncontacts	223	53	23.8	110	49.3	52	23.3	8	3.6
Females	Contacts	27	6	22.22	14	51.86	7	25.92	—	
	Noncontacts	177	54	30.5	83	46.9	35	19.8	5	2.8
Both sexes	Contacts	50	11	22.0[b]	27	54.0	11	22.0	1	2.0
	Noncontacts	400	107	26.7[b]	193	48.3	87	21.7	13	3.3

[a] All subjects were adolescents or young adults 10–18 years old.
[b] The test of standard error (S.E.) between two proportions has been applied. $Z = 0.7507$, $p > 0.05$, not significant.

at both 8 and 20 months post-vaccination. An enhanced reaction was also seen in lepromin-positive individuals at 8 months, but not at 20 months post-vaccination. Non-vaccinated subjects, both lepromin-positive and -negative, exhibited no changes in the reactivity throughout the observation period.

Serum Ab-PGL levels in healthy household contacts in this study were about 40% of the standard reference serum containing pooled LL sera. Vaccination did not result in any significant alterations (Table 8).

DISCUSSION

Leprosy has a very long incubation period and, therefore, in immunoprophylactic trials, years of observation will be required to measure the efficacy of a vaccine in terms of its capacity to lower incidence rates. As the first step, therefore, it would be essential to show that a "candidate" vaccine is able to induce persistent change in immunity. A vaccine inducing short-lived immunity is of only academic interest, especially for countries like India, where millions of people need to be vaccinated (9). In such a situation, operationally, it would be extremely difficult to repeat vaccination at short intervals.

It must be realized, however, that all forms of immunity are not protective in nature. In the case of leprosy, cell-mediated immunity (CMI) is the dominant host resistance, and antibody formation plays little role. The skin reaction to the appropriate antigens and to the lymphocyte transformation test (LTT) are the two most widely used laboratory parameters of CMI. Available clinical, laboratory, and experimental evidence shows that the late lepromin (Mitsuda) reaction is closely linked to the immune status of the host against *M. leprae*. Thus, in LL patients, who represent one end of the leprosy spectrum, the lepromin reaction is negative but their tissues are laden with *M. leprae*. On the other hand, in the paucibacillary tuberculoid patients the reaction is strongly positive (23). Pioneering work by Dharmendra and Chatterjee (12) has shown that lepromin-negative individuals

TABLE 4. *Relationship between Mitsuda reaction, BCG, and response to PPD.*

	No.	PPD (mm)		Mitsuda reaction (mm)							
		<10	>10	<3		3–5		5–7		>7	
				No.	%	No.	%	No.	%	No.	%
BCG positive	310	146	164	77	24.8	159	51.4	68	21.9	6	1.9
BCG negative	90	58	32	30	33.3	44	48.9	12	13.3	4	4.5
PPD positive (>10 mm)	196	—	—	51	26.0	87	44.4	51	26.0	7	3.6
PPD negative (<10 mm)	204	—	—	69	33.8	88	43.1	44	21.6	3	1.5

TABLE 5. *Vaccine-induced lepromin conversion in healthy noncontacts.*

Sr. no.	Age/sex	BCG	PPD (mm)	Mitsuda reaction (mm)		
				Pre-vaccination	Post-vaccination	
					8 wks	1 yr
1	13/M	+	0	0	4	5
2	14/M	+	10	1	7	7
3	13/F	−	0	1	4	6
4	14/F	+	10	1	5	ND[a]
5	15/F	+	4	1	7	5
6	16/F	+	0	1	5	7
7	17/F	+	2	1	6	6
8	13/F	+	10	1	4	6
9	14/F	+	0	5	5	5
10	14/F	−	0	1	3	ND
11	17/M	+	8	0	3	ND
12	13/F	+	5	1	3	4
13	12/F	+	0	1.5	2.5	5
14	16/F	+	5	1	0	5
15	17/M	+	2	1	0	2
16	15/M	+	2	2	2	ND
17	15/M	−	6	0	1	6
18	14/M	+	13	1	1	6
19	15/F	−	0	2	1	6
20	13/F	+	0	0	1.5	8
Total conversions (>3 mm)					9/20 (45%)	15/16 (94%)

[a] ND = not done.

in endemic areas run a very high risk of contracting the multibacillary forms of the disease. According to Job, *et al.* (17), Mitsuda-positive armadillos are relatively resistant to the disease. Leprosy has been induced recently in a variety of monkeys, in which the disease shows the pattern of tissue involvement akin to that seen in man (27). Further, the lepromatous form is seen in lepromin-negative animals. According to Bjune, the LTT is not a good indicator of protective immunity. It correlates well with cellular hypersensitivity (2). Somewhat similar conclusions could be drawn from the data of Convit, *et al.* (5). In their vaccinated patients, although skin responsiveness to antigens of *M. leprae* was persistent over the years, the LTT was only occasionally positive.

In this study, the vaccine has induced a dose-dependent lepromin conversion at 8 weeks. At the high dose, 79% of the volunteers exhibited lepromin conversion. But when the dose was reduced to about 1/30th, the conversion was seen in only 46% of the volunteers. A similar relationship was observed in the magnitude of the response. The present study shows that BCG vaccination or the tuberculin reaction has no influence on lepromin reactivity or on the rate of conversion. These observations are similar to those of Gill, *et al.* (14). An interesting feature of this study is that with increasing duration of time, even at the low dose, a very high conversion rate (>90%) was observed. It is difficult to explain this phenomenon.

Nonvaccinated, healthy lepromin-negative subjects did not exhibit any change in

TABLE 6. *Effect of lepromin test on Mitsuda-negative subjects.*

Sex	Volunteers		Mean Mitsuda reaction (range) in mm	
	No.	Age	First test	Second test
Males	11 (3)[a]	7–18	1.46 (0–2.5)	1.31 (0–2.5)
Females	13 (5)[a]	6–18	1.04 (0–3)	1.40 (0–3)

[a] Denotes the number of household contacts; the rest were from the general population.

TABLE 7. *Effect of vaccination on lepromin reaction in household contacts.*

Group	Mean lepromin reaction ± S.D.[a] (mm)					
	No. volunteers	Initial	8 mos. later	No. volunteers	Initial	20 mos. later
Vaccinated						
Lepromin positive	28	5.46 ± 2.02	8.71[b] ± 1.73	12	6.5 ± 2.64	7.91 ± 2.06
Lepromin negative	9	0.22 ± 0.66	7.33[c] ± 1.87	7	0.95 ± 0.97	5.43[c] ± 1.52
Nonvaccinated						
Lepromin positive	20	5.95 ± 2.24	5.7 ± 2.06	8	6.12 ± 1.80	5.37 ± 1.41
Lepromin negative	5	0.40 ± 0.89	0.8 ± 0.58	5	0.6 ± 1.09	1.8 ± 0.44

[a] Student's paired *t* test was applied to test the difference between initial and 8- or 20-month post-vaccination Mitsuda reactions.
[b] $p < 0.01$.
[c] $p < 0.001$.

lepromin reactivity, indicating that the conversion was not merely a consequence of the previous lepromin test. As mentioned earlier, in our study areas the patients are not segregated, and even the noncontacts (general population) are frequently exposed to *M. leprae*. As a consequence, they might have already reached the maximum response to challenges by antigenic doses of *M. leprae*. This may explain why no conversion was observed as the result of the first lepromin test. However, no change in reactivity was observed by Gill, *et al.* [14] in their preliminary trials of a vaccine containing heat-killed *M. leprae* A (armadillo-grown *M. leprae*). They had vaccinated 31 healthy adults with different doses in Norway. At the lowest dose of 1.7×10^7, which was four times the antigenic dose in the lepromin used in this study, the workers observed no change in skin reaction to *M. leprae* antigens. Norway is a leprosy-free area. These observations, therefore, indicate that heat-killed *M. leprae* at the dose of 4×10^6 bacilli/per person, which is used in the lepromin test, does not act as a microvaccine.

Tuberculoid leprosy, in which patients exhibit high CMI, is characterized by granulomatous nerve lesions [7, 23]. Many patients with borderline leprosy develop acute neuritis during reversal reactions [22, 25]. Although its exact mechanism is still not fully understood, the nerve damage is suspected to be due to hypersensitivity to intraneural *M. leprae* antigens [16, 18, 25]. This view is supported by studies of Mshana, *et al.* [19] who have demonstrated the presence of *M. leprae* antigens in the nerves of leprosy patients. Further, rabbits sensitized to *M. leprae* develop neuritis [20] when challenged with intraneural injection of *M. leprae*. In endemic areas, residents are continuously exposed to *M. leprae* infection. Due to the existence of crossreacting CMI antigens [4, 13, 15], vaccinated patients could be at major risk of developing neural lesions. This is especially so for lepromin-positive individuals who already possess a strong CMI to *M. leprae* antigens. In our studies on LL patients, no significant vaccine-induced neural lesions were observed in spite of lepromin conversion [10]. Convit, *et al.* [6] have reported similar results in their studies using a vaccine containing killed *M. leprae* plus BCG. In our study also there has as yet been no clinical evidence of hypersensitivity, and no nerve lesions have been observed.

The Ab-PGL levels in the subjects in this study were about 40% of that of the standard reference pooled lepromatous sera, although some contacts did show rather high pre-vaccination values. This is similar to that observed by Brett, *et al.* [3] in their tropical controls and by Wu, *et al.* [26] in a normal Chinese population. Vaccination did not bring about any increase in the antibody levels. As mentioned earlier, antibodies have little role in resistance against leprosy, but have been implicated in certain hypersensitivity reactions [25]. They could also form immune complexes that could suppress CMI

TABLE 8. *Effects of vaccination on circulating Ab-PGL.*

Sr. no.	Age/ sex	Lepromin reaction (mm)		PGL-antibodies[a]	
		I[b]	II[c]	I	II
8 mos. post-vaccination					
1	7/F	0	10	0.56	0.35
2	7/M	2	7	0.88	0.55
3	6/F	0	12	0.35	0.27
4	10/F	0	10	0.46	0.49
5	19/F	4	10	0.56	0.27
6	19/M	8	8	0.24	0.37
7	14/F	7	7	0.44	0.39
8	7/F	3	10	0.36	0.30
9	9/F	3	10	0.41	0.37
10	50/F	4	7	0.48	0.32
11	9/M	5	7	0.46	0.43
12	17/F	5	8	0.25	0.37
13	60/M	7	8	0.44	0.38
Mean				0.46	0.37[d]
20 mos. post-vaccination					
1	10/M	2	4	0.29	0.31
2	25/F	0	8	0.31	0.47
3	22/F	0	4	0.31	0.38
4	10/M	0	4	0.29	0.24
5	20/F	0	6	0.03	0.36
6	5/M	5	8	0.38	0.38
7	10/F	5 u[e]	6	0.47	0.36
8	22/M	5 u	12 u	0.23	0.31
9	10/M	9 u	9 u	0.34	0.56
10	12/F	5	8	0.43	0.56
11	12/F	7	6 u	0.20	0.59
12	6/F	7 u	8 u	0.43	0.69
13	29/F	12 u	10 u	0.43	0.49
14	16/M	6 u	10 u	0.26	0.43
15	28/F	9 u	8 u	0.52	0.22
16	6/M	4	5 u	0.18	0.40
17	30/F	3	6	0.27	0.50
Mean				0.35	0.42[d]

[a] Results are expressed as the fraction of the OD of the controls which varied between 1.2 and 1.5.
[b] I = initial (pre-vaccination) response.
[c] II = 8 or 20 months post-vaccination.
[d] Differences are not significant.
[e] u = ulcer.

(24). For these reasons an ideal antileprosy vaccine should not stimulate antibody formation. The ICRC vaccine meets this requirement.

Immunity induced by our vaccine persists at least up to 3 years, which is the maximum duration of observation in healthy subjects at the moment. In another study, in which 22 vaccinated LL patients were followed for 5 years, Mitsuda conversion was persistent even at the end of 5 years. The observation indicates that a single injection would induce long-lasting immunity in high-risk groups. The vaccinated subjects, including those who were initially lepromin positive, have been healthy throughout the observation period. Except for a small scar similar to that observed after BCG, there have been no untoward reactions. The vaccine is well accepted, being given as a single injection. The ICRC antileprosy vaccine, therefore, is a promising candidate vaccine. Its large-scale field trials will begin soon.

SUMMARY

Long-term effects of the administration of the ICRC antileprosy vaccine in healthy subjects have been investigated both in household contacts of leprosy patients and noncontacts in a general population. Each volunteer received a dose of vaccine containing either 0.5×10^9 or 1.7×10^7 bacilli intradermally. The vaccine induces a dose-dependent lepromin conversion in negative subjects at 8 weeks after vaccination. One year later, the conversion rates are more than 90% in both high- and low-dose groups. Lepromin conversion is stable for at least 3 years. When administered to the lepromin-positive contacts, the vaccine induces a statistically significant increase in intensity of the reaction at 6 months. During the 3-year observation period, the subjects have remained healthy and no untoward effects, including any neurological lesions, have been observed. There has also been no change in the circulating level of antibodies against the phenolic glycolipid-I antigen of *Mycobacterium leprae* as a result of vaccination. The vaccine thus induces not only stable immunity but is safe and, being given as a single injection, has a high acceptability. Its field trials will begin soon.

RESUMEN

Se investigaron los efectos a lorgo plazo de la administración de la vacuna contra la lepra ICRC, en sujetos sanos incluyendo a contactos familiares de pacientes con lepra y a personas (no contactos) de la población general. Cada voluntario recibió, intradermicamente, una dosis de vacuna conteniendo 0.5×10^9 ó 1.7×10^7 bacilos. La vacuna induce una conversión a la lepromina dependiente de la dosis en sujetos negativos a las 8 semanas de la vacunación. Un año más tarde el grado de conversión es mayor al 90% en ambos grupos de dosis alta y baja. La conversión a

la lepromina es estable por lo menos durante 3 años. Cuando se administra a los contactos lepromino-positivos, la vacuna induce un incremento estadisticamente significativo en la intensidad de la reacción, a los 6 meses de la vacunación. Durante el periodo de observación de 3 años, los sujetos han permanecido sanos y no se han observado lesiones neurológicas ni otros efectos indeseables. Tampoco han habido cambios en los niveles de anticuerpos circulantes contra el glicolípido fenólico-I del *Mycobacterium leprae* como consecuencia de la vacunación. Asi, la vacuna no solo induce inmunidad estable sino que además es segura y, aplicada en una sola inyección es altamente aceptada. Pronto comenzarán las pruebas de campo.

RÉSUMÉ

Les effets à long terme de l'administration du vaccin anti-lépreux ICRC ont été étudiés chez des sujets sains, tant des contacts domiciliaires de malades de la lèpre que des non-contacts appartenant à la population générale. Chaque volontaire a reçu une dose de vaccin contenant soit 0, 5 × 10^6, soit 1, 7 × 10^7 bacilles, par voie intradermique. Le vaccin a provoqué chez les sujets négatifs, huit semaines après la vaccination, un virage de l'épreuve à la lépromine, en rapport avec la dose administrée. Un an plus tard, les taux de virage atteignaient plus de 90%, tant dans les groupes ayant reçu une dose élevée que dans ceux qui n'avaient reçu qu'une dose faible. Le virage de la lépromine se maintient pendant au moins 3 ans. Lorsqu'il est administré à des contacts positifs à la lépromine, le vaccin entraîne une augmentation statistiquement significative de l'intensité de la réaction après 6 mois. Au cours de la période d'observation qui s'est étendue sur 3 ans, les sujets étudiés sont restés en bonne santé et n'ont présenté aucun effet secondaire, en particulier aucune lésion neurologique. On n'a pas observé de modification dans les taux circulants d'anticorps contre l'antigène phénoglycolipidique-I de *Mycobacterium leprae* suite à la vaccination. Dés lors, on peut conclure que le vaccin non seulement entraîne une immunité stable, mais qu'il est sans danger et qu'il est bien accepté lorsqu'il est administré en seule injection. Les essais sur le terrain commenceront prochainement.

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