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Immunotherapy and immunoprophylaxis of leprosy

J CONVIT, N ARANZAZU, M ZÚÑIGA, MARIAN ULRICH, MARIA E PINARDI, Z CASTELLAZZI & J ALVARADO
Instituto Nacional de Dermatología, Caracas, Venezuela

The clinico-pathologic spectrum of leprosy in its diverse forms is the expression of the immunologic response of the human host to infection by *Mycobacterium leprae*.

In this spectrum, the lepromatous pole is characterized by a total lack of cell-mediated effector immunological responses; this lack is also seen *to a lesser degree* in borderline lepromatous (BL) as well as in other forms, which present a variable degree of deficiency of this type of immunity as they are located along the spectrum towards the tuberculoid pole of the disease.

There is substantial evidence that the immunological defect which reaches its maximum expression in lepromatous patients is also present in other population groups, including a small portion of the healthy population. This evidence includes the following observations:

(a) A significant proportion of patients with the indeterminate form of leprosy show immunological behaviour similar to that of the lepromatous patient, characterized by a lack of cell-mediated manifestations toward *M. leprae*. Without adequate treatment, this indeterminate form of leprosy shows a tendency to progress to lepromatous leprosy (LL). We have seen this phenomenon of progression towards the LL pole in indeterminate patients who, after many years of treatment, have stopped their medication due to various reasons and suddenly re-appear with LL or BL disease. On the other hand, indeterminate lesions in Mitsuda-positive patients show a tendency to regress spontaneously, or to evolve towards the highly resistant forms of the disease.[1]

(b) Studies of the Mitsuda reaction in the general population, both in endemic and non-endemic areas, show that a small proportion of the general population is persistently Mitsuda-negative. In 1978 we studied the persistence of this negativity towards lepromin in the population of a non-endemic area of Venezuela and found that 1·25% of the general population remained

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persistently Mitsuda-negative after 4 injections of lepromin during an 8-month period.[2]

In 1955, Dharmendra & Chatterjee[3] reported the results of a study carried out in endemic areas in India which gave extremely interesting results. They found that among a group of 650 healthy contacts of leprosy patients, 126 were Mitsuda-negative. Of these, they tested 109 with a series of 3 applications of lepromin during the course of 1 year; a group of 16 remained negative even after the 3 lepromin injections. After 15–20 years, a new examination showed 10 active cases of leprosy among this group of 16 persons; 8 of them were lepromatous cases. No less than 55% of the lepromatous cases found in this study had developed in that 2·3% of the population which remained persistently negative after repeated lepromin injections. The other 45% appeared among those persons who were Mitsuda-negative in the first test, of whom only 1 had responded to repeated injections of lepromin; the rest came from the 17 initially negative contacts who did not receive several injections of lepromin. Not a single lepromatous case appeared in the group of 524 Mitsuda-positive contacts. These observations suggest an immunological defect in healthy persons before the development of the clinical disease, and they give the bases for the selection of high-risk populations in immunoprophylaxis studies. They also open the possibility of important studies on the mechanism of the immunological defect in persons who do not present the possible side-effects of active lepromatous leprosy and/or prolonged treatment.

After a decade of active research, it seems evident that the important immunological defect in lepromatous leprosy is a specific defect towards *M. leprae*. These patients do not present the clinical complications characteristic of generalized immuno-deficiency; apparently there is no increase in susceptibility towards viral, mycotic or malignant processes.

One of the most significant contributions toward the production of skin-test reagents for use in the immunological and epidemiological evaluation of reactivity to *M. leprae* and/or the vaccine itself has been the development of a suitable procedure for the purification of *M. leprae* from the tissues of experimentally infected armadillos. This procedure, designed to eliminate tissue contaminants while conserving the immunogenic and antigenic properties of *M. leprae*, has been developed at the National Institute for Medical Research by Dr Philip Draper, in close collaboration with Dr Dick Rees.

When using SDS-polyacrylamide electrophoresis methods to analyse a soluble extract obtained from bacilli purified according to the 1/79 IMMLEP Protocol developed by Draper, we found more than 40 proteic and polipeptidic bands, without considering the lipid and polysaccharide fractions or the insoluble fraction.

This extract produces important cross-reactions in guinea-pigs sensitized with BCG, an observation which clearly demonstrates the presence of common

antigens between *M. bovis* and *M. leprae* in this preparation. In any case, lepromatous patients *do not* recognize these common antigens, even though they recognize specific antigens of other mycobacteria. More than 65% of a group of 231 lepromatous patients give strong reactions, with an average of 30·5 mm of diameter, to 2 units of PPD. In this same group, 96% give completely negative reactions to a soluble antigen from *M. leprae*. This same extract produces reactions over 10 mm in diameter in 70–75% of the contacts tested in endemic areas of Venezuela. As we shall see later, the usefulness of this type of antigen for detecting the non-reactive population is unquestionable, but positive reactivity cannot be attributed to a response towards specific *M. leprae* antigens.

In our laboratories we are specially interested in the possible importance of a macrophage defect in the cellular alteration observed in the progressive forms of leprosy. Lepromatous macrophages possess the necessary enzymatic machinery to digest *M. leprae* if they are activated by another immunological mechanism which undoubtedly requires the intervention of sensitized lymphocytes.[4] But the importance of the macrophage is not limited to its response towards lymphocytic stimuli; these cells play a fundamental role in the processing and presentation of antigens to the lymphoid system in the induction of the immunological response, in immunosuppression, etc.

The following could be postulated: a primary defect in the processing and/or in the presentation of *M. leprae* antigens by the non-reactive macrophages would result in an inadequate immunological response, characterized by the activation of sub-populations of suppressor cells or lack of an adequate stimulus to helper lymphocytes. This possibility seems attractive when interpreting the results of immunotherapy with *M. leprae* and BCG in lepromatous patients. One of the more obvious consequences of the use of this combination is the forced digestion of *M. leprae*, a process which could produce the generation of the respective immunogens.

The concept that the immunological defect in leprosy is irreversible has been expressed frequently. This concept is backed by the observation that lepromatous patients treated successfully with chemotherapy do not develop positive reactions towards *M. leprae* even when they are stimulated by procedures which produce an immunological conversion in normal persons.[5]

The results we will see with immunotherapy with a mixture of *M. leprae* and BCG indicate that the defect is reversible in a high percentage of cases, if an adequate immunotherapeutical procedure is used. This observation gives preliminary but substantial support to the idea that prophylactic and therapeutical vaccination could offer an important option in leprosy control.

Immunotherapy

A decade ago we showed that Mitsuda-negative persons, including healthy individuals and leprosy patients, do not eliminate a suspension of heat-killed

Mycobacterium leprae from their tissues in a period of 1 to several months, but that they do eliminate BCG. In the first case, a macrophagic granuloma is formed which retains the injected *M. leprae* in its cells.[6] In the second, there is development of an immune granuloma and the BCG is eliminated. When both mycobacteria are injected together in the same site a typical immune granuloma is formed and both mycobacteria are eliminated in a short period.[4] These results suggest that the use of this mixture *M. leprae* plus BCG could provoke the digestion of *M. leprae* and the liberation of some of its antigens which could stimulate the immune system in persons normally unable to carry out this digestion. BCG would act as a stimulant of the digestive process through the activation of macrophages. Both components of the vaccine, heat-killed *M. leprae* and BCG, have been used in intracutaneous injections in human beings during many years without producing adverse side-effects, so there would be sufficient reason to expect that a mixture of both would be well tolerated by non-reactors to *M. leprae*.

Based on these considerations, in 1973 we started using a mixture of heat-killed *M. leprae* and live BCG in 6 patients with inactive lepromatous leprosy, 6 with indeterminate leprosy, Mitsuda-negatives, and in 6 persistently Mitsuda-negative contacts. In the contacts we used only one dose of the vaccine; the patients received several injections during a period of 1 or 2 years. During a 6-year observation period, we have seen important immunological changes, including the regression of indeterminate lesions and a strong and persistent positivization of the Mitsuda reaction.[7] Due to the favourable changes seen and the lack of adverse side-effects, we have proceeded to evaluate this procedure in 577 persons within the immunotherapy programme of the National Institute of Dermatology.

This group includes 25 persistently Mitsuda-negative contacts, 46 patients with indeterminate leprosy, also Mitsuda-negative, 155 patients with inactive BL and LL and 351 patients with active BL or LL. Before vaccination we did a detailed clinical, dermatological and neurological examination, biopsy of active lesions and humoral and cellular immunity tests, *in vivo* and *in vitro* (skin reactivity to PPD, to soluble antigen from *M. leprae* and to Mitsuda antigen, *in vitro* lymphocyte transformation and determination of suppressor cells and antibodies against *M. leprae* through a micro-ELISA test).

The vaccine we use contains 6×10^8 bacilli of *M. leprae* purified according to the method developed by Draper[8] from tissue of experimentally infected armadillos and killed in the autoclave at 121°C during 15 minutes, plus viable BCG in a variable amount (from 0·01 mg to 0·2 mg), according to the reaction of the patient to 2 units of PPD. The vaccine is applied intradermally in 3 sites, both deltoid regions and the upper part of the back.

Table 1 shows the patients studied, changes in the 48-reactivity towards *M. leprae* soluble antigen and clinical changes seen.

The group of 25 contacts, most of them adults, who had remained persistently

Table 1. Immunotherapy with a mixture of *M. leprae* plus BCG in leprosy groups studied and changes obtained

Classification period of vaccination	No.	Skin-test reactivity		Clinical and histopathological changes		
		SA pos. %	Mitsuda pos. %	Reduced infiltration %	Reversal reaction %	Total %
Active BL/LL						
6 mo.	18	0	—	0	8	6
18 mo.	74	20	—	27	20	47
> 19 mo.	259	38	—	43	27	71
Inactive BL/LL						
6 mo.	3	67	—			
18 mo.	39	46	—			
> 19 mo.	113	63	—			
Indeterminate						
6 mo.	2	100	—			
18 mo.	12	83	75			
> 19 mo.	32	97	88			
Contacts						
6 mo.	25	100	84			

Mitsuda-negative represents the most interesting group in relation to the potential use of this vaccine in the immunoprophylaxis of leprosy. All became positive towards *M. leprae* after 1, or rarely 2, vaccinations. The Mitsuda reaction has become positive in all who have been tested with this antigen.

We have seen important clinical, histopathological and immunological changes in the group of 46 patients with indeterminate leprosy. The clinical changes included the appearance of a papular eruption, with tuberculoid structure, which later regressed spontaneously; the lesions regressed and the hypopigmented areas became re-pigmented. A very high percentage became positive to soluble antigen and Mitsuda antigen. It was necessary to use 4 or more vaccinations to produce these changes, especially in the prelepromatous indeterminate patients who had bacilli at distant sites from their lesions (knees, ears, etc.).

As expected, it has been more difficult to produce changes in lepromatous or borderline lepromatous patients, even those who are bacteriologically negative after prolonged chemotherapy. Even in this group, 59% have responded to soluble antigen from *M. leprae* after 3 or more vaccinations.

The changes observed in active LL or BL patients who have been vaccinated 3 or more times are especially interesting. The clinical changes include reversal reactions, characterized by formation of nodules and plaques over their chronic

lesions, reactivation of lesions, better definition of borders, des-infiltration and progressive regression.

At the same time we have seen important histopathological changes, including infiltration by mononuclear cells, epithelioid differentiation in some cases and noticeable deterioration and reduction of the mycobacterial population. Of the 351 patients with active LL or BL, 62% showed some or all the changes described above; 32% became positive to soluble antigen, most of them after 5 or more vaccinations.

Most of the patients with active LL or BL presently receive chemotherapy with sulphones or rifampicin, but we have also seen the above changes in patients who are not receiving chemotherapy for various reasons (sulphone-resistance, very strong side-effects to chemotherapy or non-compliance with treatment). The immunological changes towards soluble *M. leprae* antigen have been seen very rarely in patients treated exclusively with chemotherapy.

Side-effects have been controlled with adequate treatment with thalidomide and triamcinolone and do not compare unfavourably to side-effects observed during conventional chemotherapy. In some cases with very large bacillary loads, we have observed fever and general malaise, which was controlled with small doses of corticosteroids. A very important observation has been the small number of adverse reactions in nerves. We have seen serious neuritis in only 4 cases and moderate reactions in 19 more. In 3 BL cases recently we have seen neuritic reactions of the external sciatico-popliteal nerve with Stepage (drop-foot). These side effects disappeared after 6 weeks' treatment with triamcinolone, 12 mg/day. The rest of the neuritic phenomena were managed easily with small doses of steroids, 4 mg/day, and left no permanent sequellae.

Another secondary phenomenon also seen recently is two cases of hepatitis with ictericia, also in BL cases with reversal phenomena; these cases were easily managed with 8 mg/day of triamcinolone.

The majority of secondary reactions have been observed in BL cases.

The results presented show the efficacy of the mixture of *M. leprae* and BCG to induce immunological changes in low-resistance leprosy and in Mitsuda-negative contacts. Therefore, the use of this same procedure would be justified as an immunoprophylaxis method for this disease.

Immunoprophylaxis

The bases for immunoprophylaxis as we understand it at present can be summarized as follows:

1 The population susceptible to leprosy, especially in its progressive forms, represents a very small proportion of the general population. This fact, plus the possibly high cost of producing a vaccine based on *Mycobacterium leprae* purified

from experimentally infected armadillos, justify a selective approach to vaccination, limited to high-risk populations, that is, contacts.

2 The high-risk population can be identified by two criteria, epidemiological and immunological. Through the first, we identify contacts around active leprosy cases. In Venezuela we estimate an average of 5 intradomiciliary and 45 extradomiciliary contacts per case, on the basis of previous epidemiological surveys. The interest of the group of extradomiciliary contacts lies on the fact that 75% of new cases of leprosy have been found among this group of persons. The immunological criterion used to identify high-risk contacts is a negative reaction towards soluble *M. leprae* antigen. Supposedly all contacts have been exposed to infection by *M. leprae* and those with normal immunological reactivity should have developed delayed hypersensitivity towards this organism.

3 From the evidence presented at the beginning of this presentation and the discouraging results of large-scale trials with BCG, the use of *M. leprae* or BCG alone do not offer much hope of an adequate vaccine. The favourable results obtained in the immunotherapy of leprosy with a mixture of *M. leprae* and BCG, plus the persistence of the immunological conversion seen in Mitsuda-negative contacts during an observation period of several years, indicate that this combination can represent a highly efficient vaccine.

4 The only absolute criterion to determine the efficacy of a preventive vaccine would be the incidence of new cases of leprosy; the evaluation would depend on a 5- or 10-year observation period. In any case, observations during the immunotherapy trial indicate that the induction of an immunological conversion of skin reactivity towards soluble *M. leprae* antigen can become a useful criterion to evaluate the response to the vaccine in terms of percentage of positive reactors and persistence of conversion.

Preliminary data obtained in an immunoprophylaxis trial carried out in two western states of Venezuela which are highly endemic for leprosy are extremely interesting. I will present only the most relevant results due to space limitations.

In the first stage of this trial we identified a total of 2,659 contacts in two work areas in Apure and Tachira States; 293 of these were household contacts (Figure 1 and Table 2). We examined them all clinically and neurologically, and skin tests with 2 units of PPD and 0·5 or 1·0 μg of soluble *M. leprae* antigen were applied; circulating antibodies were also studied in a micro-ELISA test.

Skin reactivity towards these soluble antigens is shown in Tables 3 and 4, according to age and sex. The reactivity to SA increases with age from the 12- to 14-year group, with an average reaction diameter less than 12 mm, up to a maximum in the 25–29-year group (20·7 and 16·9 mm in Apure and Tachira, respectively). Until this age group the correlation between age and reactivity is positive. From 40 years on, the phenomenon is inverted, reactivity is gradually reduced and the correlation between age and reactivity becomes negative.

In relation to reactivity to SA in age groups under 12 years, we did a

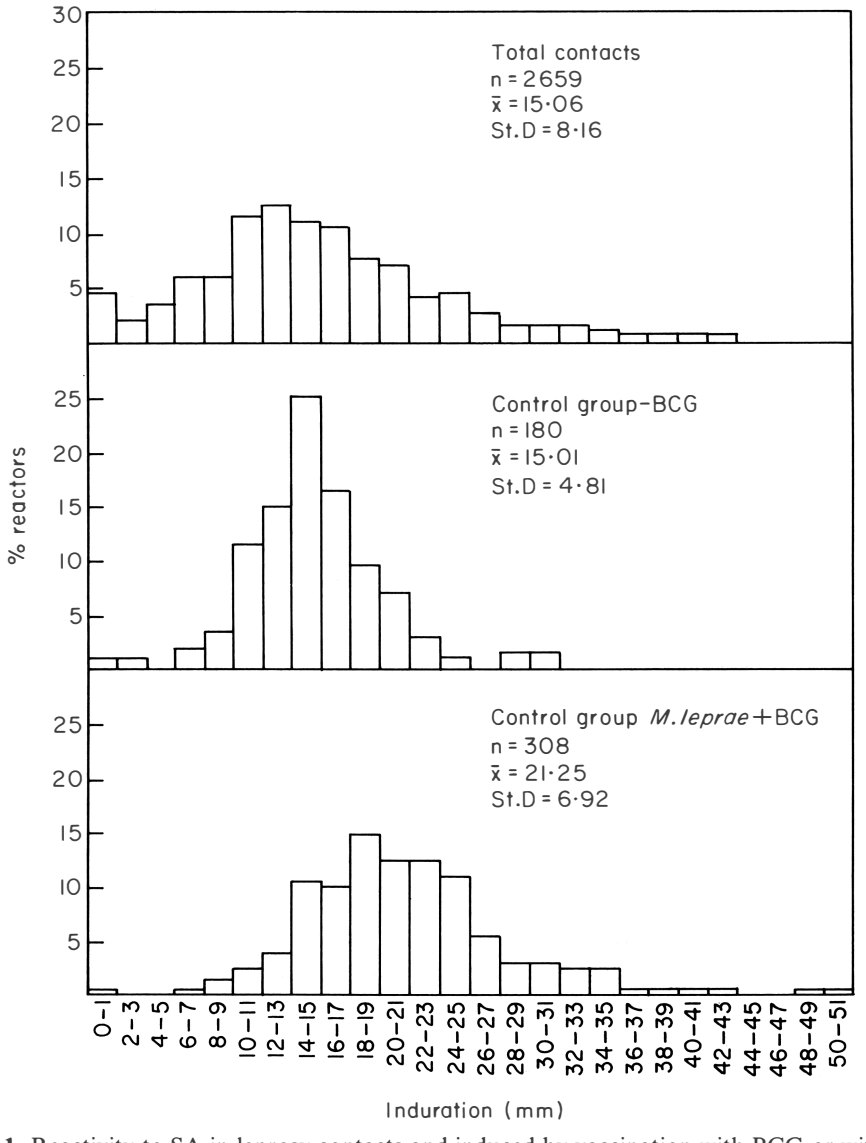

Figure 1. Reactivity to SA in leprosy contacts and induced by vaccination with BCG or with the mixture *M. leprae*–BCG in contacts initially negative. Sixty days after vaccination control, Apure–Tachira, 1981 (together).

preliminary trial with this antigen in 84 children in Apure and we saw that the 0–5 year group (30) had an average induration of 4·07 mm, with 90% under 10 mm. In children 6–11 years old the average was 10·6 mm and the proportion of 'negatives' (0–9 mm) was 40·7%.

The distribution by age and relationship with a leprosy case (Table 5), shows that in household contacts, reactivity increases earlier and apparently remains

Table 2. Immunoprophylaxis trial. Distribution of participants according to age groups. Apure and Tachira States, 1981

Age groups	Apure				Tachira			
	n	%	*N*-household	Household	*n*	%	*N*-household	Household
12–14	236	15·6	195	41	115	10·2	101	14
15–19	363	24·0	317	46	213	19·0	185	28
20–24	202	13·4	183	19	142	12·7	132	10
25–29	155	10·3	127	28	99	8·8	92	7
30–34	107	7·1	93	14	85	7·6	83	2
35–39	95	6·3	83	12	84	7·5	82	2
40–44	99	6·5	83	16	58	5·2	53	5
45–49	85	5·6	78	7	78	7·0	74	4
50–54	58	3·8	52	6	73	6·5	66	7
55–59	40	2·6	37	3	50	4·5	47	3
60–64	27	1·8	23	4	53	4·7	50	3
65–69	24	1·6	18	6	34	3·0	34	—
70 y+	21	1·4	16	5	38	3·4	37	1
Totals*	1,512	100·0	1,305	207	1,122	100·0	1,036	86

* The exact age could not be determined in 25 persons included in the study.

higher throughout. The percentage of negatives is less than that of non-household contacts in all age groups. According to this analysis, one of the groups at highest risk would be that of non-household contacts 40 years old or over, a fact which has shown an epidemiological corroboration during the preliminary stages of our trial.

We found 21·8% of 'non-reactors' in the population 12 years old or over when using criterion of induration of 9 mm or less at 48 hours (Table 6). The non-reactors were divided in two groups; one to be vaccinated with the same mixture of autoclaved *M. leprae* and BCG used for immunotherapy and the other, the control group, only with BCG.

Two months after vaccination both groups were again tested with *M. leprae* soluble antigen. As we can see in Table 6 and Figure 1, a high percentage of both groups became positive. In the group of 308 persons vacinated with the mixture *M. leprae*–BCG, we saw an average induration of 21·25 mm at 48 hours with soluble antigen; 56% of this group have strong reactions, 20 mm of induration or more and only 1·9% persisted as 'non-reactors' with reactions 9 mm or less of induration. On the other hand, the average induration in the group of 180 persons vaccinated with BCG alone was 15·0 mm; only 14% gave reactions 20 mm or more and almost 8% persisted as 'non-reactors'. This initial evaluation indicates that the *M. leprae*–BCG mixture induces an immunological conversion towards soluble antigen clearly superior to that induced by BCG alone.

Table 3. Reactivity to SA, according to age groups, in contacts. Apure and Tachira States, 1981

Age groups	Apure			Tachira		
	n	Avg Ind (mm)	% < 10 mm	*n*	Avg Ind (mm)	% < 10 mm
12–14	236	11·8	37·3	115	11·6	38·3
15–19	363	14·3	24·5	213	13·5	23·5
20–24	202	17·4	14·8	142	16·1	18·3
25–29	155	20·7	5·8	99	16·9	13·1
30–34	107	18·4	13·0	85	15·5	22·4
35–39	95	18·6	14·7	84	15·9	10·7
40–44	99	16·4	20·2	58	13·9	27·6
45–49	85	17·0	14·1	78	12·6	33·3
50–54	58	15·4	13·7	73	13·3	21·9
55–59	40	11·4	37·5	50	14·1	28·0
60–64	27	12·2	22·2	53	12·3	30·2
65–69	24	14·8	12·5	34	12·7	20·6
70 y+	21	14·4	9·5	38	12·0	26·3
Totals	1,512	15·75	20·3	1,122	14·1	23·7

Avg Ind = Average Induration

Apure:	*Age*	n	*(mm)*	*% < 10 mm*
	0–5	30	4·07	90·5
	6–11	54	10·6	40·7

Table 4. Reactivity to SA, distribution by age and sex. Apure and Tachira States, 1981

Age groups and sex		Apure			Tachira		
		n	Avg Ind (mm)	% < 10 mm	*n*	Avg Ind (mm)	% < 10 mm
12–19	Males	250	12·6	28·8	160	12·3	27·5
	Females	349	14·0	29·2	170	13·4	29·4
20–39	Males	173	18·1	12·1	134	13·8	15·7
	Females	386	18·9	11·9	276	17·2	17·0
40–59	Males	102	14·3	18·6	97	12·7	25·8
	Females	180	16·5	20·0	161	13·9	29·2
60 y+	Males	35	12·9	22·9	54	12·1	29·6
	Females	37	14·4	8·1	70	12·5	28·6
Total	Males	560	14·6	21·4	445	12·81	23·8
	Females	952	16·5	19·6	677	14·98	24·2

Table 5. Reactivity to SA antigen according to whether the contact lives in the same house with a leprosy patient or is a non-household contact. Apure and Tachira States, 1981 (summary)

Age groups	Household contacts			Non-household contacts		
	n	Avg Ind (mm)	% < 10 mm	*n*	Avg Ind (mm)	% < 10 mm
12–19	129	16·0	21·7	798	12·7	30·2
20–39	94	20·7	8·5	875	17·3	14·5
40 y+	70	17·5	12·9	668	13·8	24·1
Total	293	17·9	15·4	2,341	14·7	22·7

Table 6. 'Natural' reactivity to SA and sensitivity induced through BCG and the vaccine (*M.l.*) + BCG in contacts living in the areas selected for the trial of an antileprosy vaccine. Apure and Tachira States, 1981

Millimetres of induration	Initial population		Neg vac BCG		Neg vac *M.l.* + BCG	
	No.	%	No.	%	No.	%
0–9	579	21·8	14	7·8	6	1·9
10–14	792	29·8	73	40·6	33	10·7
15–19	622	23·4	67	37·2	97	31·5
20–24	366	13·7	20	11·1	90	29·3
25 y+	300	11·3	6	3·3	82	26·6
Total	2,659	100·0	180	100·0	308	100·0

1 'Negatives': reactions between 0 and 9 mm.
2 Second dose of SA 60 days after vaccination.

The difference in reactivity towards soluble antigen in both groups becomes much more evident at 8 months after vaccination (Figure 2 and Table 7). In the control group vaccinated with BCG, nearly 50% presented reactions 9 mm or less of induration (average of the whole group 8·99 mm); this fact shows that BCG-induced is not only weak, but also short-lived reactivity. The average of induration in the group vaccinated with the mixture was 16·32 mm at 8 months and 14% had reactions 9 mm or less. These results at 8 months support the initial conclusion obtained with the results at 2 months. It should be emphasized that the initial reactivity in the two negative groups (0–9 mm), subsequently vaccinated

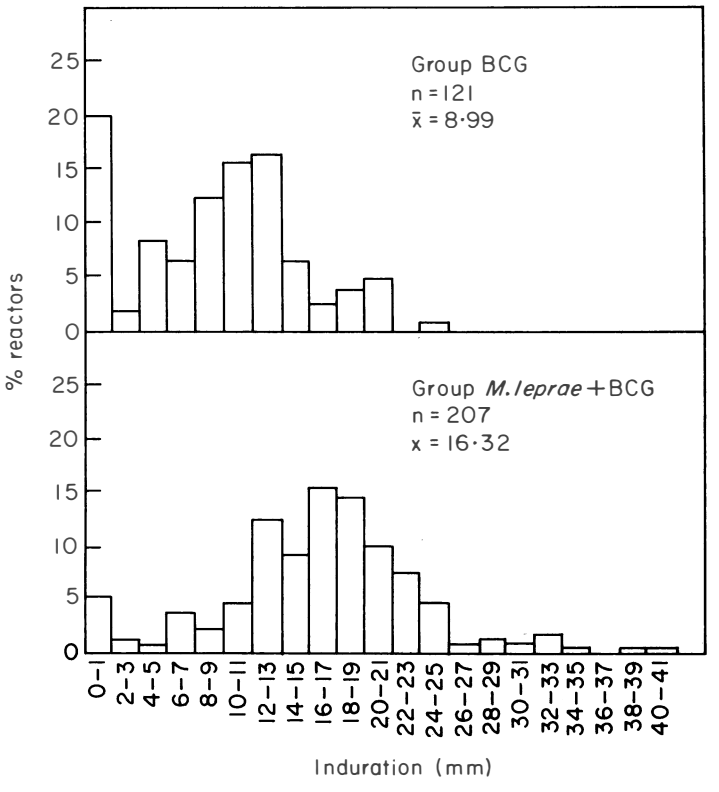

Figure 2. Reactivity to SA in leprosy contacts and induced by vaccination with BCG or with the mixture *M. leprae*–BCG in contacts initially negative. Eight months after vaccination control, Apure–Tachira, 1982 (together).

Table 7. Comparative sensitivity to SA antigen induced by BCG or *M. leprae* + BCG, at 60 days and 8 months after vaccination. Tachira and Apure States, June 1982

Millimetres of induration	Vaccination with BCG				Vaccination *M.l.* + BCG			
	60 days		8 months		60 days		8 months	
	n	%	*n*	%	*n*	%	*n*	%
0–9	14	7·8	59	48·8	6	1·9	29	14·0
10–14	73	40·6	43	35·5	33	10·7	45	21·7
15–19	67	37·2	12	9·9	97	31·5	72	34·8
20–24	20	11·1	7	5·8	90	29·3	41	19·8
25 y+	6	3·3	—	0·0	82	26·6	20	9·7
Total	180	100·0	121	100·0	308	100·0	207	100·0

Table 8. Initial reactivity of the negative contacts to SA and modifications induced by vaccination with BCG or *M. leprae* plus BCG, 2 and 8 months after vaccination

	Control group (BCG)			Test group (*M. leprae* + BCG)		
Evolution	*n*	$\bar{X}$ (mm)	SD	*n*	$\bar{X}$ (mm)	SD
Initial reaction	180	5·01	3·07	308	4·93	3·15
Skin test, 60 days	180	15·01	4·81	308	21·25	6·92
Skin test, 8 months	121	8·99	6·06	207	16·32	7·28

with BCG or with *M. leprae* and BCG, was of 5·01 and 4·93 mm average diameter, respectively, with very similar standard deviations (3·07 and 3·15 mm) (Table 8).

The future stages of this trial contemplate the repetition of the clinico-dermatological examination and skin tests with soluble *M. leprae* antigen at yearly intervals during 5 years and less frequent examinations in the next 5 years. The preliminary results have already become the basis for the protocol of a much larger trial in Venezuela, which will include the study of 61,000 contacts.

The results of the immunotherapy and immunoprophylaxis programmes developed in Venezuela offer the hope of new methods for antileprosy campaigns, at a moment when the alarming increase of sulphone-resistance makes it necessary to intensify the search for a new approach to solve this problem.

Before closing I would like to make some comments on the group of persons who, after being vaccinated with the mixture, appear as negatives (0–9 mm) or weakly positive (10–14 mm) after an intradermal test with soluble antigen.

A not yet determined percentage of these negative persons have high titres of circulating antibodies with the micro-ELISA test. This situation is similar to that seen in patients with low-resistant forms of leprosy (LL, BL) and Mitsuda-negative contacts, and we have considered the possibility that this group may require more than one vaccination to obtain favourable immunological changes.

According to these last considerations, the above mentioned group of non-reactors would be the root of the leprosy endemia. Therefore, the schedule of antileprosy campaigns could be the following: contact population divided in reactors and non-reactors to soluble antigen, vaccination with the *M. leprae*–BCG mixture and later re-vaccination of the persistently non-reacting group. With this approach the antileprosy campaign would be secondary to the immunotherapy of 'non-reactors' after the first dose of vaccine, since by producing immunological changes in this population group we would prevent the creation of new infected foci and, therefore, the maintenance of the endemia.

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