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# Immunotherapy with a Mixture of *Mycobacterium leprae* and BCG in Different Forms of Leprosy and in Mitsuda-negative Contacts[1]

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The treatment of low-resistance forms of leprosy presents a series of important problems because of several contributing factors. The insidious multiplication of a microorganism possessing limited intrinsic toxicity in a host which is incapable of mounting an adequate immunological response to that organism results in a chronic disease requiring prolonged treatment. The response to chemotherapy is slow, even in adequately treated cases. Relapses occur in a significant number of arrested cases when treatment is suspended or abandoned; lifelong treatment is indicated in lepromatous and borderline lepromatous leprosy. Oral chemotherapy with dapsone (DDS) is difficult to control and often irregular, particularly after several years of continuous treatment. As a consequence of irregular treatment and the use of inadequate doses of DDS, sulfone-resistance has become a significant problem. This complication usually becomes manifest as secondary resistance in the treated patient, but a significant number of cases of infection with sulfone-resistant bacilli (i.e., primary resistance) have been reported ([10]). These observations represent potentially serious obstacles in the control of leprosy. Combinations of therapeutic drugs clearly offer the possibility of circumventing some of the difficulties described above, but are totally inaccessible to the vast majority of patients with leprosy; the possibility of extending chemoprophylaxis to a significant number of contacts is economically inconceivable in the foreseeable future.

These difficulties have created the urgent need to develop a new therapeutic procedure, directed toward the modification of the pathogenic basis of the low-resistance forms of leprosy.

Considerable evidence has been accumulated in our laboratories and in others concerning the nature of the immunologic defect in low-resistance forms of leprosy. The defect appears to be highly specific for *Mycobacterium leprae*; non-specific immunologic depression may occur as a sec-

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ondary phenomenon in some cases of advanced lepromatous leprosy, but there is no evidence that this phenomenon contributes significantly to the evolution and pathology of the disease ([4, 19, 20]). It is generally accepted that the defect may exist prior to contact with *M. leprae*, and that successful chemotherapy of the disease and apparent cure is not accompanied by the development of cell-mediated immunity to the microorganism.

In 1972, we demonstrated that Mitsuda-negative contacts as well as lepromatous patients were unable to eliminate heat-killed *M. leprae* from skin sites in a period of one or two months; these same individuals developed a competent immune granuloma to other mycobacteria such as BCG ([3]). In marked contrast, Mitsuda-positive individuals rapidly eliminated *M. leprae* and developed granulomas with a tuberculoid structure at the injection sites.

Two years later, we reported that the simultaneous injection of heat-killed *M. leprae* and BCG in these non-reactors induced the formation of an immune granuloma, with elimination of both mycobacteria. The apparent macrophage defect in the non-reactors, reflected in the inability to digest *M. leprae*, had been overcome by the injection of the mixture ([5]). These experiments provided the experimental basis for the use of the mixture of two mycobacteria, one of which provides the necessary specific antigens and one of which triggers macrophage digestion, in studies of immunotherapy and immunoprophylaxis in leprosy and in apparently healthy individuals who constitute a high risk group in view of their failure to develop cell-mediated immunity to *M. leprae* in spite of prolonged contact with the disease ([2, 6]). The use of a mixture of two mycobacteria to enhance resistance to the pathogenic member of the pair was studied in murine leprosy more than a quarter of a century ago by Hanks and Fernandez ([9]). Transitory induction of Mitsuda reactivity by the injection of BCG in some groups of patients with leprosy, not associated with protective effects, has been reported in the literature ([15]). In our experience, repeated injections of BCG or lepromin alone do not induce persisting immunological responses in these groups of non-reactors.

In the present study, we have evaluated immunotherapy with the mixture of heat-killed *M. leprae* and viable BCG in four groups of individuals:

a) Persistently Mitsuda-negative contacts of patients with leprosy—apparently healthy individuals who are weak or non-reactors to *M. leprae*.
b) Mitsuda-negative patients with indeterminate leprosy, in the early stage of disease but with a lepromatous potential because of their condition of being non-reactors.
c) Lepromatous and borderline lepromatous patients who are bacteriologically negative after sulfone treatment, in whom effective immunization would prevent relapse and the creation of new foci of infection.
d) Patients with active lepromatous and borderline lepromatous leprosy.

In this communication we will emphasize clinical and histopathological changes produced in patients and the possible mechanisms of activation by this mixture; immunological and bacteriological observations will be presented in detail in subsequent publications.

## MATERIALS AND METHODS

**Cases.** The 529 cases studied include the following groups:

a) Twenty-five contacts of lepromatous patients who were persistently negative reactors to the injection of standard Mitsuda antigen ($1.6 \times 10^7$ acid-fast bacilli/0.1 ml), even after several vaccinations with BCG.
b) Forty-one patients with indeterminate leprosy, who presented multiple hypochromic lesions with alterations in sensitivity; isolated *M. leprae* were detected in the small cutaneous nerves of the lesions in the majority of these cases. These patients were Mitsuda-negative after three or four applications of standard lepromin and two or three vaccinations with BCG.
c) One hundred nine patients with borderline lepromatous (BL) or lepromatous (LL) leprosy, bacteriologically negative after prolonged treatment with sulfones.
d) Three hundred fifty-four patients with active BL and LL.

The procedures included in this study were approved by the Ethical Committee of the Instituto Nacional de Dermatología and the competent authorities of the Ministry of Health and Social Assistance. Consenting individuals were included only after full explanation of the purposes and possible risks involved. Patients with extensive active forms of the disease were only incorporated after several years of observation had been completed in groups of patients with incipient or arrested disease.

All the individuals studied were given a complete dermatologic, neurologic, ophthalmologic and general clinical examination. Patients with active disease were classified according to the criteria of Ridley and Jopling ([14]). Biopsies for histopathologic evaluation were stained for cellular studies by hematoxylin-eosin, and the Fite-Faraco technique was used for the demonstration of mycobacteria.

Preliminary immunological evaluation included skin tests with standard lepromin, soluble antigen prepared from *M. leprae*, and two units of PPD. Blood samples were taken for *in vitro* immunological studies to be described elsewhere.

Soluble antigen (SA) was prepared from *M. leprae* purified from experimentally infected armadillo tissues by the Draper protocol ([7]). The purified bacilli were partially disintegrated by passage through a French pressure cell (Aminco) at 10,000 lbs/in$^2$; bacillary debris was removed by centrifugation. The supernate was filtered through an Amicon PM 30 membrane; the low molecular weight filtrate was collected and sterilized by filtration through a Millipore membrane. Protein content was determined by the Lowry method ([12]); the solution was diluted to 10 $\mu$g protein/ml with sterile phosphate-buffered saline, pH 7.2, bottled and autoclaved at 121°C for 15 min. Endotoxin is not detectable in this material by the *Limulus* lysate test (Difco PYROTEST®), and primary toxicity has never been observed in guinea pigs nor in human volunteers. Skin tests were performed by the intradermal injection of 0.1 ml of this extract in the volar surface of the forearm; induration was measured at 48 hr and reactions greater than 10 mm in diameter were considered positive.

**Immuno-stimulation.** Patients and contacts were injected with a mixture of *M. leprae* obtained from the tissue of experimentally infected armadillos, purified by the Draper protocol and heat-killed by autoclaving at 121°C for 15 min and viable BCG (Institut Pasteur, France). Studies by Shepard, *et al.* ([16]), as well as many years of experience with Mitsuda antigen, have demonstrated that the immunogenicity of *M. leprae* is not detectably altered by autoclaving. Each vaccine contains $6 \times 10^8$ *M. leprae* and variable amounts of BCG, depending upon the individual's previous response to 2 units of PPD, in a volume of 0.5 ml; the freshly reconstituted BCG is added just prior to vaccination. The mixture is injected intradermally in three sites in the deltoid regions and upper back.

The relationship between the size of the reaction to PPD and the amount of BCG included in the vaccine is as follows:

| Reaction to 2 units PPD at 48 hr | BCG |
|---|---|
| 0–9 mm | 0.1–0.2 mg |
| 10–14 mm | 0.1 mg |
| 15–24 mm | 0.04 mg |
| 25 mm or greater | 0.02 mg |

Standard treatment with dapsone (1.5 mg/kg/day), injectable sulfone (3 cc Hansolar®/month) or rifampin (600 mg/day) was continued in 204 of the patients with active BL or LL. Chemotherapy was not continued in the other 150 persons in this group for one or more of the following reasons. a) Previous compliance with treatment was extremely irregular and unreliable or had been abandoned altogether; b) chronic lesions after many years of regular treatment suggested the presence of sulfone-resistant strains of *M. leprae*, confirmed in two such cases in mouse foot pad tests carried out by Dr. Charles Shepard; c) conventional chemotherapy produced adverse secondary reactions or was accompanied by intractable reactional episodes; and d) the patients were new cases who had never been treated by chemotherapy.

Patients were examined at regular intervals of from one to four weeks, depending upon their disease status. During the course of the study, and as a consequence of clinical modifications such as partial regression of lesions or change in the position in the

TABLE 1. *Clinical and immunological changes in leprosy patients and contacts, untreated after vaccination.*

| | LL | | BL | | Indeterminate leprosy (IL) | Contacts |
|---|---|---|---|---|---|---|
| | Active | Inactive | Active | Inactive | | |
| Total studied | 168 | 32 | 63 | 8 | 29 | 25 |
| Clinical changes | 110 | NA[a] | 51 | NA | 17 | NA |
| Positive reaction to SA | 32 | 4 | 39 | 4 | 28 | 25 |
| No change | 58 | 28 | 12 | 4 | 1 | 0 |

[a] NA = Not applicable.

clinical spectrum, biopsies were taken for histopathological evaluation; blood samples were taken for *in vitro* immunological tests; and skin tests were performed with SA and PPD. Patients who did not show significant changes by clinical, histopathologic and immunologic criteria were re-vaccinated at intervals of two to three months. Clinical criteria are not easily subjected to quantitative evaluation, and in large part depend upon the coincidence of independent judgments by experienced clinicians. Histopathological specimens were independently evaluated in parallel studies by one of us (O.R.). Responses of less than 10 mm of induration at 48 hr to SA, or fluctuations around this limit, represent the immunologic criterion for re-vaccination.

## RESULTS

Tables 1 and 2 show the distribution of contacts and patients studied, and the principal changes observed.

The group of 25 persistently Mitsuda-negative contacts represents the most interesting group with regard to the potential use of this vaccine in the immunoprophylaxis of leprosy. All 25 individuals showed positivization of their reactions to SA (i.e., reactions greater than 10 mm in diameter) after one or, rarely, two vaccinations. The 23 contacts who were retested with standard Mitsuda antigen had become positive within 8 to 12 weeks after vaccination. Although some persistently Mitsuda-negative contacts undoubtedly harbor infection with *M. leprae*, which might become manifest as tuberculoid or borderline tuberculoid leprosy in the presence of cell-mediated immunity ([18]), no such case has appeared in this small group of individuals. The rapid response to immune stimulation in these individuals suggests that the immunological defect in these individuals is minimal and easily overcome.

In the group of 41 Mitsuda-negative patients with indeterminate leprosy, important clinical, histopathological and immunological changes have occurred. The clinical and histopathological changes are observed after two or three vaccinations within a period of six months. Clinical changes include the appearance of an eruption formed by multiple small papules and, in a single case, of a typical tuberculoid plaque. Re-pigmentation of hypochromic

TABLE 2. *Clinical and immunological changes in leprosy patients in whom chemotherapy was continued after vaccination.*

| | LL | | BL | | Indeterminate leprosy (IL) |
|---|---|---|---|---|---|
| | Active | Inactive | Active | Inactive | |
| Total studied | 111 | 53 | 12 | 16 | 12 |
| Clinical changes | 49 | NA[a] | 6 | NA | 4 |
| Positive reaction to SA | 25 | 23 | 5 | 11 | 12 |
| No change | 52 | 30 | 6 | 5 | 0 |

[a] NA = Not applicable.

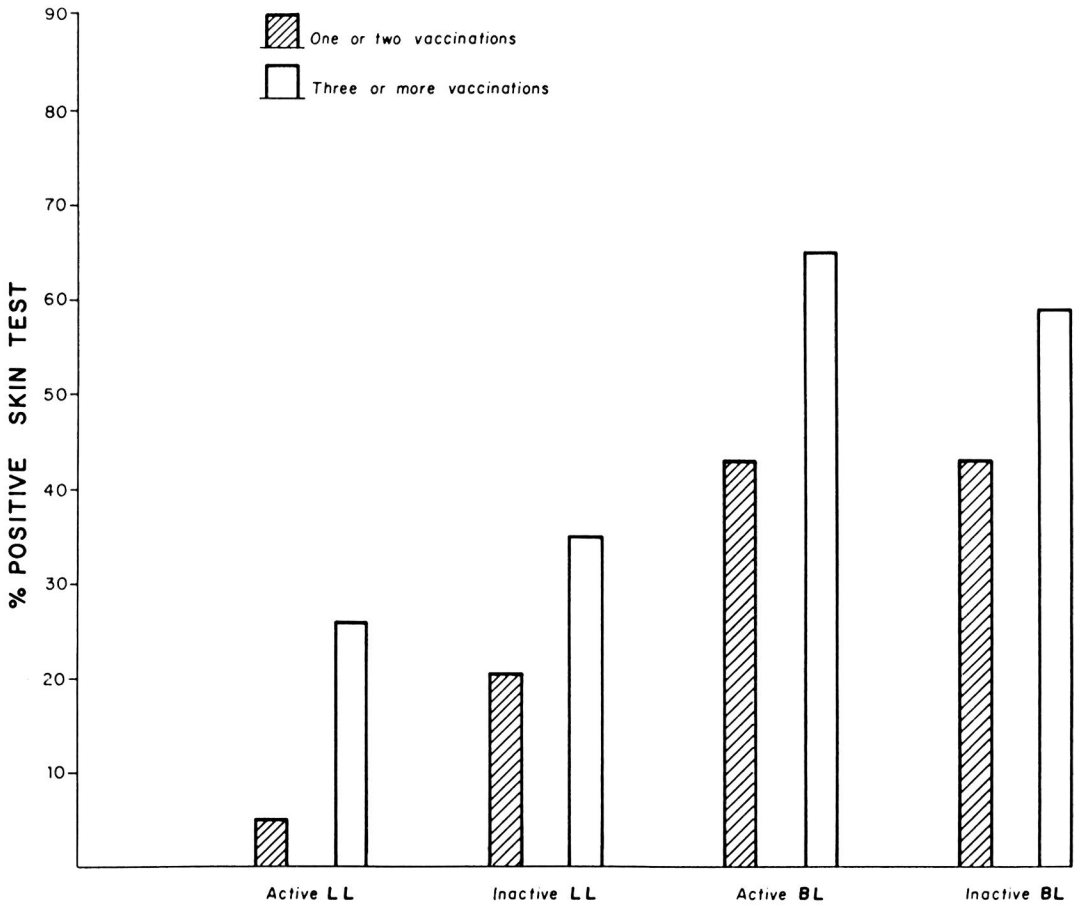


FIG. 1. Conversion of skin tests reactions to 1 μg SA from *M. leprae* after immunotherapy with *M. leprae* + BCG.

lesions is frequent but requires two or three years. Histologic study of the papular lesions shows a follicular tuberculoid structure. The papules disappear spontaneously after three or four months. Thirty-nine patients gave positive reactions to SA and 33 to Mitsuda antigen. In eight patients, a final Mitsuda reaction has not been applied. Four vaccinations were required to produce the changes described in those patients who showed progressive evolution toward lepromatous leprosy prior to vaccination, as indicated by the presence of bacilli at sites distant from the lesions (ear lobes, knees). Although early tuberculoid and indeterminate leprosy show a tendency toward spontaneous regression, especially in children, this tendency is correlated with Mitsuda reactivity ([11]). We have not observed this phenomenon in adults with persistently Mitsuda-negative indeterminate leprosy.

As expected, it has been more difficult to induce immunologic changes in LL or BL patients, even in those who are free of clinical lesions after prolonged treatment with sulfones. Nevertheless, in this latter group of 109 individuals, 42 have become immunologically reactive to SA after one to six vaccinations (Fig. 1).

The changes observed in the group of active BL and LL patients are of extraordinary interest. The clinical changes include reactivation of lesions and sharper definition of their borders, formation of nodules and plaques superimposed on chronic lesions, and progressive flattening.

Parallel to the clinical changes observed, important histological changes also oc-

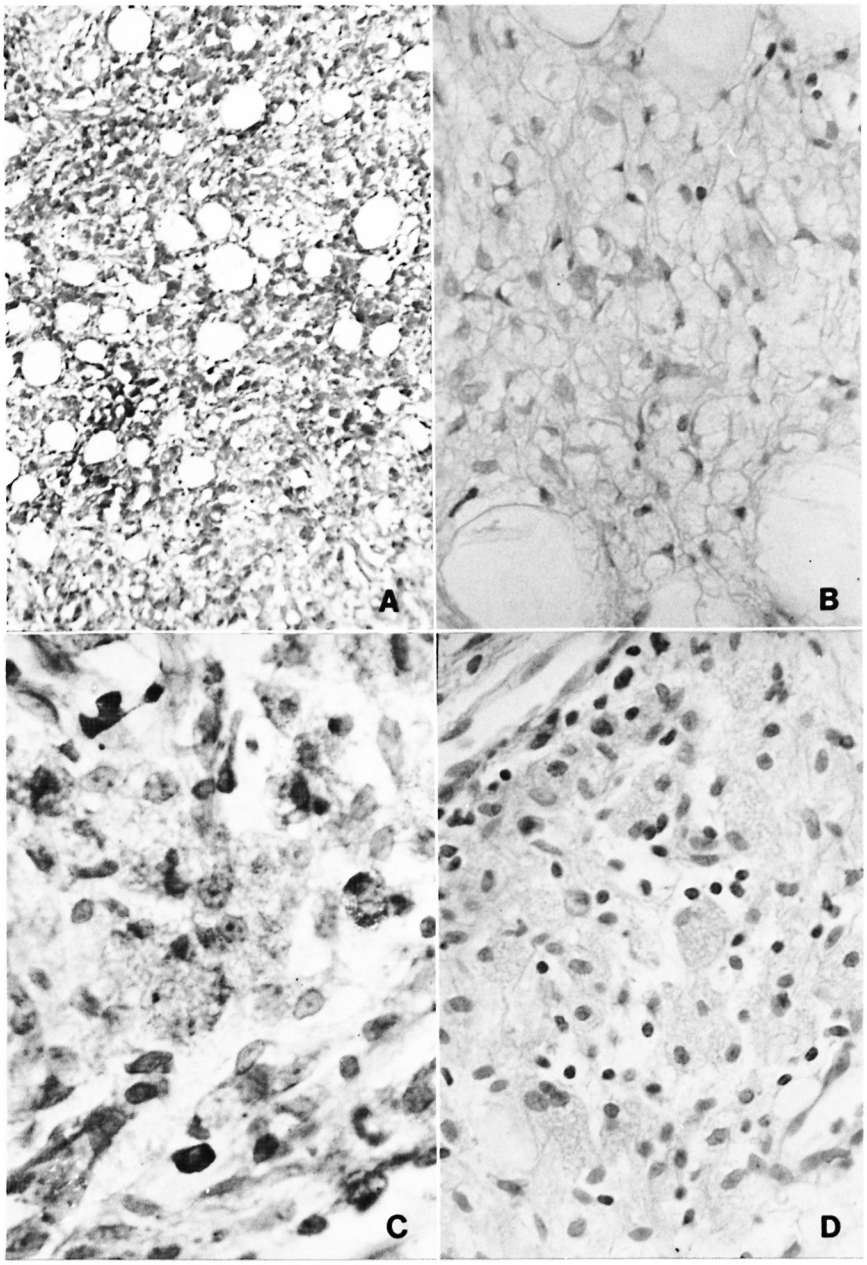


FIG. 2. Biopsies taken from patient H.H. with lepromatous leprosy before immunotherapy (A = Fite-Faraco; B = hematoxylin-eosin) and after four vaccinations (C = Fite-Faraco; D = hematoxylin-eosin) with the mixture of *M. leprae* and BCG. (Magnification: A = ×128; B, C, and D = ×320.)

curred. These changes are of two types which depend upon the degree of macrophage compromise in the granuloma. In lesions formed by highly vacuolated, bacilliferous macrophages, the most striking observation after vaccination is the accumulation of numerous, relatively small macrophages around foci of these cells and around the small vessels of the granuloma. These small macrophages may become parasitized by *M. leprae* during the early stages of vaccination. Lymphoid elements are ini-

tially scarce, but become more numerous as the period of observation increases. Increased lymphocyte infiltration is accompanied by disintegration of the acid-fast bacilli and disappearance of the highly vacuolated macrophages forming the original granuloma. This type of change is shown in Figure 2. The masses of acid-fast bacilli present before vaccination are no longer visible after four vaccinations. The hematoxylin-eosin stain of the same biopsy reveals the presence of lymphoid elements. The clinical response in this type of lesion is a relatively slow flattening of the lesions, which may require 18 months or more. Side effects of fever and malaise are not frequent.

In other cases in which the macrophages bear a smaller parasitic load and do not show extensive vacuolization, lymphoid cells become relatively numerous in the lesions after vaccination, and epithelioid differentiation as well as bacillary disintegration are often observed within a period of 12 months. Fever and general malaise are more frequent in these cases than in those described above.

Of a total of 256 patients with active BL and LL vaccinated three or more times, 180 (70%) have shown some or all of the clinical and histopathological changes described above (Fig. 3). Eighty-seven have become SA positive. A lower percentage of BL and LL patients who have received only one or two vaccinations show important changes, but even in this group 37% have presented clinical changes and 14% have become SA positive (Figs. 1 and 3).

The clinical, histopathological and immunological changes in an appreciable number of the active BL-LL group are of a magnitude sufficient to permit their reclassification in the leprosy spectrum toward a position which reflects greater resistance. Some of the changes reported above, such as progressive flattening of lesions, are observed over a course of several years' treatment by chemotherapy. Others, however, such as the sharp definition of borders, marked epithelioid differentiation in the granuloma, and reactivity to soluble antigen from *M. leprae*, have not been observed in patients treated exclusively by chemotherapy in our Institute. There were no apparent significant differences in the

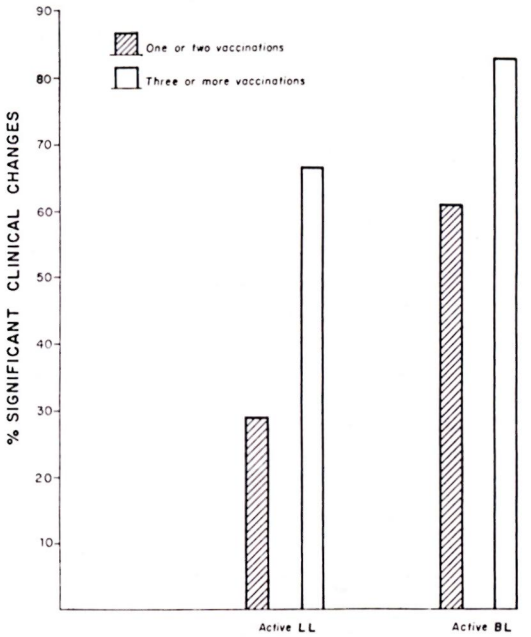


FIG. 3. Percentage of patients with active leprosy showing significant regression of clinical lesions after vaccination with *M. leprae* BCG. Groups include 75 patients with LL and 23 with BL vaccinated once or twice; 204 LL and 52 BL vaccinated three or more times.

appearance of these changes between the individuals who received both immunotherapy and chemotherapy and those who received immunotherapy alone.

**Secondary reactions.** Some of the patients with lepromatous leprosy included in the study presented reactions of erythema nodosum leprosum (ENL) prior to or at the time of vaccination. These lesions, characterized by extensive infiltration by polymorphonuclear leukocytes (PMN), probably represent Arthus reactions elicited by the formation of immune complexes *in situ* ([21]). Histopathological evaluation is often necessary to distinguish these lesions from the erythematous lesions produced by mechanisms of cell-mediated immunity, and failure to make a clear distinction may lead to erroneous interpretations of clinical observations. In the cases studied here, vaccination coincided with a disappearance of ENL lesions in some patients. In others, typical ENL lesions characterized by vascular dilatation, edema and PMN infiltration were slowly and progressively re-

placed by papular lesions with extensive perivascular lymphoid cell infiltration. The complete transformation of ENL lesions to the lymphoid type occurred within a period of one year or more. During this period febrile episodes ceased and the remarkable response to thalidomide, which is characteristic of ENL, was lost.

The transformation of undifferentiated macrophagic granulomata into lesions with epithelioid differentiation and infiltration by lymphoid elements is accompanied by an enormous reduction in the bacterial population. In these cases we have observed fever and general malaise, easily controlled by small doses of corticosteroids.

An observation of enormous importance which cannot be over-emphasized is the low number of reactions in nerve trunks, in spite of the important response at the cutaneous level. If it were not for this phenomenon and if nerves were affected with the same intensity as the skin, vaccine therapy would not be possible in bacilliferous leprosy. We have observed severe neuritis in only four of the total of 354 cases of active BL and LL studied, and moderate neuritic reactions in 19 others. These reactions, consisting of pain in somewhat enlarged cubital (ulnar) and sciatic (common peroneal) nerves, were rapidly controlled by thalidomide or corticosteroids and left no persisting sequellae.

Seven patients with active disease developed new lesions, after the second vaccination, which were characterized by the formation of macrophagic granulomata with minimal vacuolization. Globi formed by solid bacilli were present in these new lesions. Subsequent vaccinations were accompanied by the transformation of these lesions into structures similar to tuberculoid granulomata, with massive reduction in the bacillary population.

Five patients developed edematous reactions in the dorsum of the hand, with an accumulation of fluid in the synovial sac at the level of the wrist. Occasionally after vaccination, patients complained of generalized pruritis which sometimes persisted for three or four months and then disappeared spontaneously. None of the secondary reactions of these types was severe nor caused disability.

## DISCUSSION

The development of persisting reactions of cell-mediated immunity to antigenic components of *M. leprae* toward which the individuals studied were previously non-reactive, subsequent to the injection of a mixture of heat-killed *M. leprae* and viable BCG, is a phenomenon of extraordinary importance in terms of the clinical management of active leprosy and of immunoprophylaxis in the control of this disease. In addition, the concept that lepromatous leprosy is an incurable disease, based on the observation that bacilli persist after many years of treatment, perhaps in part because these patients never develop cell-mediated immunity to *M. leprae*, must be re-examined in terms of these results.

The precise nature of the immunological defect in lepromatous leprosy has not been clarified. Evidence has been presented which implicates both macrophages ([1]) and lymphoid cells ([8]) as the defective elements, and the intervention of suppressor cells has also been invoked ([13]). The fact that lepromatous patients do not develop evidence of cell-mediated immunity to *M. leprae* after prolonged treatment and apparent cure has led to speculation that this group has such a profound immunological defect that no response to specific immunotherapy could be expected. The present study clearly demonstrated that these patients do in fact respond to an adequate immunological stimulus. The reported failure to induce positive skin reactions in four inactive lepromatous patients injected with a mixture of *M. leprae* and BCG ([17]), apart from obvious considerations regarding the size of the group, may be attributed to the use of an inadequate dose of *M. leprae*, as well as the application of a single injection.

The development of a population of lymphocytes sensitized to *M. leprae* is closely associated with the clinical and histopathological changes observed, as indicated by lymphocyte infiltration in the lesions which acquire tuberculoid characteristics, and by the positivization of skin reactions of delayed hypersensitivity. Several possibilities exist to explain this phenomenon, including the following:

a) The macrophages in non-reactors are

incapable of processing and presenting antigens of *M. leprae* to lymphoid cells in an appropriate form. Simultaneous injection with BCG, to which the macrophages are or rapidly become activated, corrects this defect.

b) The vaccination procedure alters the conditions of immunomodulation, so that a population of suppressor cells is eliminated and active expression of lymphocyte activity occurs.

In this study, the majority of the patients were re-vaccinated at intervals of two or three months. One patient with advanced LL who abandoned immunotherapy as well as chemotherapy after two vaccinations was re-incorporated into the study after seven months and showed extraordinary clinical improvement. This suggests that the process of sensitization, once initiated, is self-perpetuating and may not require as many or as frequent re-vaccinations in some patients as we originally contemplated.

Two parameters are fundamental in the evaluation of the program of immunotherapy in patients with BL and LL; these are the number of vaccinations and the period of time that has elapsed since the first vaccination. In advanced cases several vaccinations, tentatively estimated as an average of six, are clearly required, and a period of observation between 20 and 24 months.


## SUMMARY

A total of 529 weak or non-reactors to *M. leprae*, including Mitsuda-negative contacts and patients with leprosy, were vaccinated once or repeatedly, as necessary, with a mixture of $6 \times 10^8$ purified, heat-killed *M. leprae* and 0.01 mg to 0.2 mg of viable BCG. Clinical, histopathological and immunological criteria were used to evaluate the response of these individuals. Clinical changes, including sharper definition of borders and progressive flattening and regression of lesions, were observed in 57% of the active LL cases and 76% of the active BL cases. Histopathological study revealed infiltration of the lesions by mononuclear cells, appearance of epithelioid differentiation, and fragmentation of the microorganisms. Delayed-type skin tests with soluble antigen from purified *M. leprae* became positive in significant numbers of each group studied. These results demonstrate the efficacy of combined immunotherapy in low-resistance forms of leprosy and potential utility in the immunoprophylaxis of the disease.

## RESUMEN

Se vacunó un total de 529 reactores débiles y no reactores al *M. leprae*, incluyendo a contactos Mitsuda negativos y a pacientes con lepra, con una mezcla de $6 \times 10^8$ *M. leprae* muertos por calor y 0.01–0.02 mg de BCG viables. Se aplicaron una o varias dosis según fue necesario. La respuesta de los individuos se evaluó de acuerdo a criterios clínicos, histopatológicos e inmunológicos. Los cambios clínicos, incluyendo una clara definición de los bordes, un aplanamiento progresivo y la regresión de las lesiones, se observaron en el 57% de los casos BL activos. El estudio histopatológico reveló una infiltración de las lesiones por células mononucleares, aparición de formas epitelioides, y fragmentación de los microorganismos. Las pruebas dérmicas de tipo tardío con antígeno soluble derivado del *M. leprae* purificado llegaron a hacerse positivas en un número significante de individuos dentro de cada grupo. Estos resultados demuestran la eficiencia de la inmunoterapia combinada en las formas de lepra de baja resistencia y su utilidad potencial en la inmunoprofilaxis de la enfermedad.

## RÉSUMÉ

Un total de 529 personnes ne réagissant pas à *M. leprae*, ou n'y réagissant que faiblement, comprenant des contacts Mitsuda-négatifs et des malades atteints de lèpre, ont été vaccinés une fois, ou de façon répétée, ainsi qu'il a été jugé nécessaire, avec un mélange de $6 \times 10^8$ de *M. leprae* tués à la chaleur, associés avec 0,01 à 0,2mg de BCG viable. On a eu recours à des critères cliniques, histopathologiques, et immunologiques, pour évaluer la réponse de ces individus. Chez 57% des cas LL actifs et chez 76% des cas BL actifs, on a assisté à des modifications cliniques, dont une meilleurs accentuation du bord des lésions, ainsi qu'un applatissement graduel et une régression de ces lésions. Les études histopathologiques ont révélé une infiltration des lésions par des cellules mononucléaires, de même que l'apparition d'une différenciation épithélioïde et la fragmentation des microorganismes. Des épreuves cutanées du type retardé, pratiquées au moyen d'un antigène soluble obtenu à partir de *M. leprae* purifié, sont devenues positives chez un nombre significatif de chacun des groupes étudiés. Ces résultats prouvent l'efficacité d'une immunothérapie combinée dans les formes de lèpre à faible résistance, et suggèrent qu'une immunoprophylaxie de cette maladie pourrait être utile.


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