Immunology, Epidemiology and Social Aspects of Leprosy
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INVESTIGATIONS ORIENTED TOWARD THE DEVELOPMENT OF A VACCINE AGAINST LEPROSY

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Leprosy is a chronic, endemic, infecto-contagious disease which affects the most heavily populated areas of the world. The global number of patients with leprosy in the world is estimated at 14 to 15 million people; 90% of these patients are found in the Asiatic and African continents. Introduction of leprosy into the Americas was relatively late, occurring in the post-Columbian period. At present, it constitutes an important public health problem in Brazil and Colombia and, on a smaller scale, in Peru, Argentina, Bolivia, Paraguay, Venezuela and the Guianas. Autochthonous cases have not been reported from continental Chile. In Europe, small residual foci persist in the Balkans, Russia, Italy, Spain and Portugal.

In endemic areas, leprosy represents an important health problem not only because of its extreme chronicity, but because of the elevated percentage of physical incapacity which it causes, varying from 30 to 50% of the cases. The heavy burden of prejudice associated with leprosy compounds its physical consequences.

The campaign against leprosy has been based fundamentally on early diagnosis of the disease, public health education and treatment of cases. This approach requires the development of an extensive infrastructure in the field, which must be maintained for long periods of time; its effectiveness is measured in terms of decades of labor. This has frequently led to the abandonment of leprosy control programs because of a lack of sustained effort and continuity.

In the last two decades, two phenomena have been clearly demonstrated which exercise a negative effect in control programs: (a) the development of secondary resistance to *Mycobacterium leprae* and, subsequently, the appearance of cases of primary resistance. This problem is undoubtedly the consequence of the use of sulfone monotherapy in irregular and often insufficient doses during the last forty years. (b) Important internal migration of the population of countries with endemic leprosy from rural areas to urban centers. This leads to the possibility of the creation of new foci of infection, which can only be controlled by modifications in present leprosy control programs. This problem is compounded when migration is not limited to internal movements of the population, but includes migration to bordering countries.

The establishment a decade ago of the Special Program for Research and Training in Tropical Diseases (TDR) by the UNDP/ World Bank/WHO has changed the perspectives for leprosy control. The IMMLEP program has defined two high priority objectives, which are the development of a preventive vaccine for leprosy and the development of simple procedures for early diagnosis which can be easily adapted to field conditions. To achieve these goals, an intensive scientific program has been developed which includes research in the areas of immunology, epidemiology, molecular biology and genetics.

As a chronic, endemic, infectious disease, leprosy possesses singular characteristics which must be considered in the development of a vaccine. First, though the infection of the population in endemic areas is very widespread, only a relatively small percentage of that population is susceptible to the development of progressive clinical disease. In terms of percentages, approximately 80% of the population possesses a high degree of resistance and does not develop progressive disease. The susceptible group who does not develop resistance to infection by *M. leprae* is found in the remaining 20% of the population. The number of new cases per year (incidence) is generally quite low, rarely exceeding 2 to 4 per thousand, while the number of accumulated cases (prevalence) shows considerable variation, to 5, 10, 20 and even 30%, depending upon the intensity of the endemicity.

Another important characteristic of leprosy is the fact that patients with progressive disease and susceptible healthy individuals show a defect in the phenomena of cell-mediated immunity (CMI), which appears to be highly specific and limited to *M. leprae*. This observation is widely accepted by investigators at present, and contrasts sharply

with the opinion widely expressed in the scientific literature during the sixties, which considered the defect to be generalized and non-specific, altering reactivity to various antigens. This aspect is of particular interest in the development of a vaccine, since the problems confronted in vaccine development presumably would be much greater if the defect were non-specific and generalized.

Our activities toward the development of an anti-leprosy vaccine have covered four stages.

The first stage was to develop a test to detect the defect in CMI which affects non-reactors, a group which includes patients with borderline lepromatous and lepromatous leprosy, persistently Mitsuda-negative patients with indeterminate leprosy and persistently Mitsuda-negative contacts. This group represents a scale of intensity in the immunological defect which progresses from the Mitsuda-negative contacts to the BL-LL group. The test developed to detect this defect consisted of the intracutaneous injection of 0.1 ml of human Mitsuda antigen containing 6.4×10^8 *M. leprae*/ml and subsequent histological evaluation of the granulomatous response [1].

This injection induced the formation of a nodule 6 to 8 mm in diameter at three weeks in the group of non-reactors to standard Mitsuda. Histological study showed that the nodule consisted of a granulomatous lesion formed by non-differentiated macrophages containing numerous intact intracellular bacilli. In contrast, the reactions in positive reactors were characterized by the development of tuberculoid-type immune granulomata and essentially complete elimination of the bacterial population. This test, then, permitted the visualization of the immunologic defect in CMI toward *M. leprae* in the group of non-reactors described above. A similar phenomenon has been observed in parasitic disease (leishmaniasis) and in deep mycoses toward their respective micro-organisms. Therefore, this type of response appears to characterize non-reactors with a CMI defect toward any parasite. The specificity of this defect is demonstrated by the fact that the group of non-reactors to *M. leprae* described above developed characteristic immune granulomata subsequent to the injection of other mycobacteria, such as BCG.

In this first stage, the response of the group of non-reactors to *M. leprae* was evaluated when they were injected with a mixture of killed *M. leprae* and live BCG [2]. In these experiments, the formation of an immune granuloma and the clearance of both mycobacteria were observed.

In the second stage of our research, we considered the possibility that the development of an immune granuloma subsequent to the injection of the mixture of *M. leprae* and BCG in non-reactors might result in the liberation of active immunogens that could induce specific CMI reactivity to *M. leprae* in these individuals. An immunotherapy protocol using the mixture of killed *M. leprae* and live BCG was applied to a group of six patients with inactive lepromatous leprosy, six with persistently Mitsuda-negative indeterminate leprosy and two Mitsuda-negative contacts (Table 1). This small group of 14 persons was observed from 1973 to 1983 and the following observations were made: the six LL patients became permanently Mitsuda-positive after 6 to 8 vaccinations, with reactions of 5 to 9 mm.; they have been without treatment for 6 to 10 years without suffering relapse.

The Mitsuda-negative patients with indeterminate leprosy were vaccinated 4 to 6 times. They all developed a papular rash with a tuberculoid structure, which disappeared after three months; one developed a plaque with a tuberculoid structure super-imposed on an old hypo-pigmented lesion of the right elbow, which also disappeared in three to four months. All became persistently Mitsuda-positive, with reactions of 6 to 12 mm.

The persistently Mitsuda-negative contacts became reactive to Mitsuda antigen (6 and 10 mm) after a single dose of immunotherapy in one and two doses in the other.

The control examinations made in this group between 1980 and 1983, after six to ten years without chemotherapy, showed all of the LL and indeterminate patients to be free of lesions.

The third stage of investigation, based on the promising results obtained with immunotherapy in the small group described above, consisted of immunotherapy in a group of 626 persons, of whom 481 had BL or LL disease, 61 with Mitsuda-negative indeterminate leprosy, 57 BB-BT cases and 27 Mitsuda-negative contacts.

Immunotherapy consisted of the repeated intradermal injection of a mixture of 6×10^8 purified, autoclaved *M. leprae* and from 0.02 to 0.2 mg. of viable BCG, depending upon reactivity to PPD. Vaccination was made in three sites on the deltoid regions and the upper back. A maximum of eight to ten injections were administered at two to three month intervals, depending upon the immunological, clinical and bacteriological changes observed.

Table 2 shows the clinical and immunological changes observed in the 626 persons who received from 1 to 10 vaccinations in the course

TABLE 1. — *Immunotherapy with the mixture* M. leprae *plus BCG in patients and contacts. Initial experience.*

Classification	Positivization Soluble Antigen				Lepromin 30 Days			
	Initial		Final		Initial		Final	
	Nº Persons	Average Induration mm	Nº Persons	Average Induration mm	Nº Persons	Average Induration mm	Nº Persons	Average Induration mm
LL	6	0	6	15,8 (12–22)	6	0	6	7 (5–9)
IL Lepromin neg.	6	0	6	16,1 6–36	6	1,3 (0–3)	6	10 (6–12)
Contacts Lepromin neg.	2	0	2	12,5 (10–15)	2	2,5 (2–3)	2	8 (6–10)

TABLE 2. — *Immunotherapy with the mixture* M. leprae *plus BCG in patients and contacts.*

Classification	Total Nº Persons	Positivization I. D. Tests				Histological Reversion Phenomena		Bacteriologically Negative	
		Soluble antigen		Lepromin					
		Nº	%	Nº	%	Nº	%	Nº	%
Active BL-LL	300	141	47,0	91	30,3	90	30,0	194	64,6
Inactive BL-LL	181	107	59,0	94	51,9	—	—	—	—
Active BB-BT	26	23	88,4	19	73,0	8	30,7	12	46,0
Inactive BB-BT	31	16	51,6	28	90,3	—	—	—	—
IL	61	58	95,0	58	95,0	—	—	—	—
Contacts	27	27	100,0	23	85,0				

of three to four years of observation. All of the patients simultaneously received chemotherapy with two or three drugs (DDS-rifampycin-Lampren).

The local reactions as well as the residual scar at the injection sites of immunotherapy were similar to the responses observed with BCG vaccination alone. Secondary reactional phenomena and neuritic reactions were no different from those which are observed in patients receiving chemotherapy alone.

The analysis of the results given in Table 2 shows that 185 of a total of 481 patients with BL or LL leprosy became Mitsuda-positive. In the other groups (BB-BT and indeterminate leprosy) the number of positive Mitsuda reactions was much higher. Of the 27 contacts, 23 gave positive reactions and the other 4 could not be tested because they did not remain in control. Biopsies were taken of the Mitsuda reactions from 55 of the BL-LL patients chosen at random; histological examination revealed the presence of an immune granuloma of variable intensity with complete clearance of the bacilli injected in the test.

The characteristics of the reversal phenomena observed are of particular interest, since they represent one aspect of the changes in CMI observed in these patients, the majority of whom had LL or BL leprosy. It should be borne in mind that these phenomena are exceptional in patients of this type who receive chemotherapy alone.

The clinical and histological forms of the reversal reactions were highly variable, and included the observation of histological changes characteristic of reversal reactions without apparent clinical modification.

Reversal reactions were of variable duration and usually were not accompanied by systemic manifestations. In exceptional cases, systemic reactions required the administration of corticosteroids for a period of six to ten weeks (4 to 8 mg. Dexamethasone). In 7 cases we observed a type of reversal reaction characterized by edema of the dorsum of the hands, feet and lower legs, which we interpret as the localization of the reaction in lymphatic vessels and nodes.

The influence of the reversal phenomena on the evolution of the disease was striking, and included regression of lesions — nodules, plaques and spots — with a notable and progressive reduction in the bacterial population. In this sense, these reversal reactions have a significance quite different from those few observed in cases which have received prolonged chemotherapy, and in which chronic lesions are very limited or do not exist. For these reasons, we consider reversal

phenomena during immunotherapy to represent a process which is of moderate duration of a few weeks and which is accompanied by notable improvement in the disease.

Positive reactions to soluble antigen of *M. leprae* were observed in 248 BL-LL cases, which represent 52%. Positivization of the reaction to soluble antigen corresponded in nearly all cases to the positivization of reactivity at 48 hours and 30 days to Mitsuda-type antigen. In some instances, however, we observed a positive 48-hour reaction to Mitsuda antigen and a negative reaction to soluble antigen and vice versa. These differences could be due to antigenic differences in the two preparations, since cell-wall antigens may be of more importance in Mitsuda-type preparations and cytoplasmic antigens in soluble antigen.

The fourth stage of our studies has been concerned with the possible use of the mixture of killed *M. leprae* and live BCG in the immunoprophylaxis of leprosy. The arguments to support the use of this mixture in preventive vaccination are as follows: (1) The evident immunotherapeutic effect in LL, BL and persistently Mitsuda-negative indeterminate leprosy, characterized by the induction of important immunological reactivity toward *M. leprae* in these patients. (2) The conversion of persistently Mitsuda-negative contacts to strong reactors, after a single vaccination in the majority of cases. (3) The immunological changes induced by vaccination with the mixture of *M. leprae* and BCG are stable; our observations include a small group of patients and contacts in which these changes have persisted for ten years.

From an epidemiological point of view, the use of a vaccine against leprosy in endemic areas is of particular interest in those groups who have an elevated risk of infection; that is to say, in contacts. In addition, it is of particular interest to identify that subgroup which is susceptible to the development of progressive forms of the disease among contacts, since that group is largely responsible for the maintenance of the endemic.

A series of studies was carried out in order to broaden the universe of contacts and to try to detect the susceptible individuals within this universe. The group of contacts was extended to include not only those living in the same household, but also non-household contacts, including relatives, friends, frequent visitors and frequently-visited households, school companions, co-workers, etc. In the

epidemiological study of contacts in endemic areas of Venezuela, an average of 50 contacts were identified for each leprosy patient; five were household and 45 non-household contacts. This type of systematic study of both types of contacts has been referred to as epidemiological screening.

The identification of the susceptible subgroup of contacts was studied subsequent to the development of a soluble antigenic extract for use in 48-hour skin tests, prepared from *M. leprae* purified by the Draper 1979 protocol from the tissues of experimentally infected armadillos. The rupture of *M. leprae* is brought about by eight passages of the bacillary suspension through a French pressure cell at 10,000 lbs/in^2; subsequent ultrafiltration gives the fraction with a molecular weight of less than 30,000 which is used.

The intra dermal 48-hour skin reaction produced by this soluble antigenic extract has been determined in 700 patients with BL and LL leprosy and is negative in more than 99%. The soluble antigen has been used in 2634 household and non-household contacts, 12 years of age or older, in the areas where immunoprophylactic studies are being carried out in Venezuela (Apure and Táchira states).

The results of this study are shown in Table 3. The proportion of negative reactions shown in the last column of this Table (21.8%) is of particular importance in our program of immunoprophylaxis, which will include some 70,000 persons from the endemic area, since the protocol is based upon the selection of negative reactors to soluble antigen for vaccination and control [3].

TABLE 3. — *Reaction to SA in contacts of leprosy patients in relation to Age.*

Age group	n. ex.	Reactions to SA	
		Avg. induration (mm)	% "negatives" 0–9 mm
12–19 years	927	13.1	29.2
20–39 years	969	17.6	13.8
40 and more	738	14.1	23.2
Total	2,634	15.05 mm	21.8%

The capacity of this antigen to identify non-reactors among patients also applies to persistently Mitsuda-negative contacts. In these contacts, we have observed discordance between the reaction to PPD, which may be strongly positive, and the negative reactions to soluble antigen. When the positive reactions to soluble antigen were studied in contacts, there was correlation to PPD reactivity in those contacts who had a BCG vaccination scar, demonstrating that the soluble antigen detects cross reactivity to BCG. In non-reactors (LL, BL and Mitsuda-negative indeterminate leprosy, Mitsuda-negative contacts), strong positivity to tuberculin PPD is frequently associated with soluble antigen negativity. It is clear that both the specific and cross-reacting antigens of *M. leprae* are involved in the absence of reactivity in non-reactors.

Another aspect of interest from the epidemiological point of view is the study of antibodies to the glycolipid isolated by Brennan as well as soluble antigen in the sera of the group of non-reactors to soluble antigen. In preliminary studies using an ELISA test to measure antibodies to phenolic glycolipid I in 300 contacts who did not react to soluble antigen in skin tests, about 3% had exceptionally high titers. This suggests the presence of a significant bacterial population of *M. leprae*, and would correspond to an incidence of about 3 per thousand in the total population studied.

Another aspect of this study which may be of importance is the bacteriological examination of skin smears in that group of persons who give negative skin tests to soluble antigen and have high levels of antibody to glycolipid. This examination might permit the early diagnosis of LL or BL clinical disease at a stage when clinical manifestations are not yet apparent.

In the preliminary study of 2634 contacts, the 570 non-reactors to soluble antigen were divided into two groups; 360 were vaccinated with the *M. leprae*-BCG mixture and 210 received BCG alone.

The responses to soluble antigen at 60 days, 8 months and 14 months after vaccination in these two groups are shown in Table 4. A statistically significant difference is observed between the percentage of negative reactors persisting after BCG vaccination (7.8%) and after vaccination with the mixture (1.9%) at 60 days. This difference increases at six months (49.2 and 13.7%, respectively) and remains relatively stable at 14 months (42 and 19.4%). Not only were there differences in the percentage of reactors observed, but the average size of positive reactions was significantly greater in the *M. leprae*-BCG

TABLE 4. — *Response to SA induced by vaccination with the mixture* M. leprae + *BCG or BCG in contacts initially "negative" (comparative evaluation).* Táchira and Apure States 1981–1982

Stages and Groups	n	mm. Induration		% Reactions (mm.)		
		Average	Std. Dev.	0–9	10–14	15 y +
Group BCG						
Initial reaction	210	5.0	3.0	100.0	0.0	0.0
60 day control	180	15.0	4.8	7.8	40.6	15.6
8 month control	118	8.9	5.7	49.2	35.6	15.2
14 month control	69	10.8	9.1	42.0	20.3	37.7
Group vaccinated M. leprae + BCG						
Initial reaction	360	5.0	3.1	100.0	0.0	0.0
60 day control	308	21.2	6.9	1.9	10.7	87.4
8 month control	204	16.3	7.2	13.7	22.1	64.2
14 month control	93	17.5	9.5	19.4	10.8	69.8

group. The significant number of reactions of 15 mm or greater at 6, 8 and 14 months suggests an important persistence of *M. leprae*-specific reactivity in the group vaccinated with the mixture. The results demonstrate that the responses to BCG were much weaker and less persistent than the response to the *M. leprae*-BCG mixture.

These preliminary results, showing a significant response to *M. leprare*-BCG vaccination in a population of persons who appear to be at particularly high risk of developing progressive disease by epidemiologic and immunologic criteria, form the basis for an immunoprophylaxis trial in more than 60,000 contacts in Venezuela, which may provide the foundation for the addition of a new and powerful element to the control of leprosy.

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