Immunosuppression and Cellular Immunity Reactions in Leprosy Patients Treated with a Mixture of *Mycobacterium leprae* and BCG[1]

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Leprosy patients present different clinical, histological, and immunological forms of disease throughout a wide spectrum. The benign form, tuberculoid leprosy, is characterized by an active response measured *in vitro* by a T-cell proliferation assay. In the multibacillary malignant form, lepromatous leprosy, T cells do not proliferate in the presence of specific and crossreacting antigens of *Mycobacterium leprae* ([6]).

The basic immunologic defect(s) in lepromatous leprosy producing reduced or absent cellular immunity to *M. leprae* is as yet not fully understood ([7]). Assays for suppression of the immune response by suppressor T cells or monocytes in lepromatous leprosy patients have been presented ([9]). Exposure of T cells and monocytes from patients with lepromatous, but not tuberculoid, leprosy to Dharmendra lepromin preparations or to *M. leprae* phenolic glycolipid ([8]) suppressed the *in vitro* mitogenic response of their lymphocytes to concanavalin A. However, other assays to detect disease-related suppression in lepromatous leprosy have provided conflicting results ([1, 13, 16]).

The purpose of this study was to evaluate the effect of treating lepromatous patients with a mixture of *M. leprae* and BCG on the reactivity of their lymphocytes in the suppressor-cell assay described by Mehra, *et al.* ([9]). In addition, proliferative responses to diverse preparations of *M. leprae* and *in vivo* responses of untreated and treated patients have been compared in this study.

[1] Received for publication on 5 March 1987; accepted for publication in revised form on 12 June 1987.

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MATERIALS AND METHODS

Isolation of mononuclear cells. Mononuclear cells were obtained from 20 ml of heparinized blood from lepromatous patients and normal volunteers by centrifugation on Ficoll-Hypaque gradients ([2]). After three washes, the cells were resuspended in RPMI 1640 (GIBCO Laboratories, Grand Island, New York, U.S.A.) with 10% of a pool of human AB serum, 100 U penicillin, 100 μg streptomycin, and 2 μmol glutamine/ml. The cells were cultured at 2×10^5/0.2 ml medium in microtiter plates. The lepromatous patients were studied before initiation of immunotherapy and after having received two to seven vaccinations with a mixture of *M. leprae* and BCG ([4]) in proliferation and skin test assays; suppression assays were performed before immunotherapy and after 2 years (eight to ten vaccinations).

Suppression assay. For the development of this study, we used the same experimental conditions employed by Mehra, *et al.* ([9]). The antigen used in immunosuppression was Dharmendra lepromin (prepared by Dr. M. Abe, National Institute for Leprosy Research, Tokyo, Japan). Concanavalin A (ConA; Sigma Chemical Co., St. Louis, Missouri, U.S.A.) 2 μg/2×10^5 cell and 10 μl of Dharmendra lepromin were added. The cultures were incubated in 95% air-5% CO_2 for 3 days. They were treated with 1 μCi ^{3}H-thymidine (specific activity 1 Ci/mole) 18 hr before harvesting, and the cells were processed for liquid scintillation.

The percentage of suppression was calculated as follows:

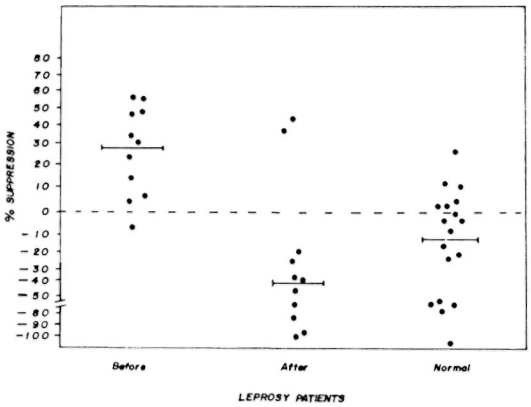
$$\%S = 100 - \frac{\text{counts per minute (CPM) ConA + lepromin}}{\text{cpm ConA}} \times 100$$

Lymphocyte proliferation assays. The antigens used were: a) 20 μl of soluble extract of *M. leprae*, 250 μg protein/ml; b) 20 μl purified *M. leprae*, 60 × 10^6 bacilli/ml; c) 20 μl heat-killed bacillus Calmette-Guérin (BCG) (Connaught Laboratories Ltd., Willowdale, Ontario, Canada), 0.18 mg/ml; d) 10 μl Dharmendra lepromin. The soluble extract of *M. leprae* was prepared by rupture of bacilli purified from the tissues of experimentally infected armadillos by the Draper protocol ([5]) by repeated passage through a French pressure cell, centrifugation at 49,000 × *g* × 1 hr at 4°C to eliminate bacillary debris, and filtration through a millipore membrane, pore size 0.45 μm. The same antigens were utilized after immunotherapy with the *M. leprae*-BCG mixture ([4]). The skin test antigens used were 2 IU PPD and 0.1 ml of a low molecular weight fraction of the soluble extract (filtrate passing an Amicon PM-30 membrane) containing 5 μg protein/ml (SA-Mlep).

Patients. The patients were seen in the Instituto de Biomedicina, Caracas, Venezuela. All leprosy patients were skin biopsied and classified according to the Ridley-Jopling criteria ([15]).

RESULTS

Our principal interest in this study was to evaluate the suppression mechanism induced to ConA by Dharmendra antigen as reported by Mehra, *et al.* and to study possible variations after immunotherapy. The Figure shows that lepromatous patients studied before treatment showed a suppression which averaged 24% in comparison with volunteer donors whose value was −20.43%. The leprosy patients treated with a mixture of *M. leprae* and BCG and observed after 2 years showed a highly significant change in the percentage of suppression (−43.84%).



THE FIGURE. Suppression percentages in leprosy patients before and after treatment with a mixture of *M. leprae* and BCG.

In relation to cellular immunity to different specific antigens of *M. leprae* and BCG, Table 1 shows lymphocyte transformation and cutaneous tests in leprosy patients before treatment with a mixture of *M. leprae* and BCG. We observed negative reactions to *M. leprae* in lepromatous patients in contrast to tuberculoid patients; a significant number of the LL-BL group reacted to BCG in transformation tests. Similar results were observed in cutaneous tests with SA-Mlep and PPD.

Table 2 shows a larger group in which the same tests were performed in leprosy pa-

TABLE 1. *Lymphocyte transformation and cutaneous tests in leprosy patients before treatment with a mixture of* M. leprae *and BCG.*

Patients not vaccinated	Lymphocyte transformation test (Stimulation index)[a]			Cutaneous tests (mm)[a]	
	M. leprae		BCG	PPD	SA–*M. leprae*
	Purified	Soluble extract		2 units	5 mg/ml
BL-LL (N = 16)	0.87[b] ± 0.03	1.0 ± 0.54	0.98 ± 0.36 (N = 11) 8.13 ± 7.75 (N = 5)	0 (N = 9)[c] 26 ± 16.0 (N = 7)	0
BT-TT (N = 8)	2.84 ± 1.48	5.07 ± 3.37	4.12 ± 2.94	20.4 ± 6.73	28.6 ± 15.48

[a] Positive values: Stimulation index = >2; Cutaneous test = >10 mm at 48 hr.
[b] Average value ± standard deviation.
[c] Positive and negative groups.

TABLE 2. *Lymphocyte transformation and cutaneous tests in leprosy patients after treatment with a mixture of* M. leprae *and BCG.*

Patients	Lymphocyte transformation test (Stimulation index)[a]			Cutaneous tests (mm)[a]	
	M. leprae		BCG	PPD	SA–*M. leprae*
	Purified	Soluble extract		2 units	5 mg/ml
			Vaccinated		
BL-LL (N = 24)	2.22[b] ± 2.11	4.50 ± 3.08	6.91 ± 6.30 (N = 18)[c] 1.43 ± 0.26 (N = 6)	33.6 ± 25.2 (N = 23) 0 (N = 1)	24.0 ± 18.31 (N = 13) 0 (N = 11)
IL[d] (N = 8)	1.85 ± 1.25	2.39 ± 0.45	5.78 ± 3.28	19.6 ± 7.5	21.33 ± 9.24 (N = 4) 0 (N = 4)
			Not vaccinated		
BT-TT (N = 8)	2.84 ± 1.48	5.07 ± 3.37	4.12 ± 2.94	20.4 ± 6.73	28.6 ± 15.49

[a] Positive values: Stimulation index = >2; Cutaneous test = >10 mm at 48 hr.
[b] Average value ± standard deviation.
[c] Positive and negative groups.
[d] IL = indeterminate leprosy.

tients treated with *M. leprae* plus BCG. An increase in the average responses to *M. leprae* antigens was observed in lepromatous patients. Of the two *M. leprae* preparations tested, soluble extract gave higher values. Both lymphocyte transformation with BCG and cutaneous reactivity to PPD also increased in patients receiving immunotherapy with the mixture.

DISCUSSION

There is a defective immunologic response in many leprosy patients to *M. leprae* antigen that is not yet well understood. Two groups of investigators have reported suppressor mechanisms involving mononuclear cells, including T lymphocytes and macrophages in leprosy ([8, 10, 13]), but their results differ with regard to the clinical form of leprosy with suppressor activity.

The results of this study confirm the presence of suppressor cells in lepromatous leprosy in the type of assay described by Mehra, *et al.* This type of suppressor-cell activity disappeared during the course of immunotherapy using a mixture of *M. leprae* and BCG in 9 of 11 patients.

We have grouped patients with borderline lepromatous and lepromatous leprosy together in this study because no significant differences were observed in their initial reactivity in any of the assays used. The clinical response after vaccination has been discussed in detail elsewhere ([4]), and the results reported in this paper do not address many of the questions related to the number of vaccinations required to induce immunological changes, clinical correlation, and other aspects of the response to immunotherapy ([3]). Suppressor responses induced by purified intact *M. leprae* or by the soluble extract of *M. leprae* did not show the same pattern of suppression as that induced by Dharmendra lepromin (data not shown).

Recently, Mehra, *et al.* ([8]) have demonstrated that a unique antigen of *M. leprae*, phenolic glycolipid-I, is also capable of inducing a suppressor response *in vitro* in the lymphocytes of patients with lepromatous leprosy. The T cells involved in this suppressor response apparently recognize and respond specifically to the terminal trisaccharide portion of this molecule.

Other assay systems ([11, 12, 14]) using diverse techniques have provided evidence for postulated suppressor mechanisms in lepromatous leprosy.

Elimination of suppressor activity as assayed in the system used in this study and positivization of the lymphocyte transformation and cutaneous tests to *M. leprae* in a significant number of BL-LL patients after

immunotherapy with a mixture of *M. leprae* and BCG confirm the immunological modulation induced by this procedure.

SUMMARY

Suppressor reactivity was studied in a group of leprosy patients before and after immunotherapy with a mixture of *Mycobacterium leprae* and BCG. The treatment increases the responses in lymphocyte transformation tests to levels which are comparable to those observed in BT-TT patients and reduces suppressor activity.

The soluble extract of *M. leprae* appears to be more sensitive than purified intact bacilli in the lymphocyte transformation tests, but this preparation did not induce suppressor reactivity with the regularity observed when using a Dharmendra preparation.

RESUMEN

Se estudió la actividad supresora en un grupo de pacientes con lepra antes y después de inmunoterapia con una mezcla de *Mycobacterium leprae* y BCG. El tratamiento incrmenta las respuestas en las pruebas de transformación de linfocitos a niveles comparables a los observados en pacientes BT-TT y reduce la actividad supresora.

El extracto soluble de *M. leprae* parece ser más adecuado que el bacilo intácto purificado en las pruebas de transformación de linfocitos, pero esta preparación no induce actividad supresora con la regularidad observada cuando se usa una preparación bacilar tipo Dharmendra.

RÉSUMÉ

Dans un groupe de malades de la lèpre, on a étudié le pouvoir suppresseur avant et après immunothérapie par un mélange de *Mycobacterium leprae* et de BCG. Le traitement a renforcé les réponses aux épreuves de transformation lymphocitaire, qui ont alors atteint des niveaux comparables à ceux observés chez des malades BT-TT; le traitement a également réduit le pouvoir suppresseur.

L'extrait soluble de *M. leprae* est apparu plus sensible que les bacilles purifiés intacts lors des épreuves de transformation lymphocitaire. Cette préparation n'a toutefois pas entraîné une activité du type suppresseur, d'une manière aussi régulière que celle notée lorsqu'on utilise la préparation de Dharmendra.

Acknowledgments. We thank Dr. B. Bloom for the Dharmendra lepromin and C. Santaella and S. Telles for preparation of the purified *M. leprae* antigen.

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