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T lymphocyte reactivity of leprosy patients and healthy contacts from a leprosy-endemic population to delipidified cell components of *Mycobacterium leprae*

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Summary In this study, we measured *in vitro* proliferative responses of peripheral blood mononuclear cells from both leprosy patients across the clinical spectrum and also healthy contacts from a leprosy-endemic population to delipidified cell components of *Mycobacterium leprae* (DCC) and Dharmendra lepromin. Dharmendra lepromin was poor in inducing *in vitro* T cell proliferation in all the study groups, even though it elicited marked *in vivo* skin test reaction in tuberculoid leprosy patients and healthy contacts. In contrast, Dharmendra preparation of BCG induced marked T-cell response in tuberculoid as well as bacterial index negative lepromatous patients. DCC induced a significantly higher lymphoproliferative response than Dharmendra lepromin in all study groups. A significant positive correlation was observed between the lymphoproliferative responses to DCC and BCG. The present study, based on a large number of leprosy patients and healthy contacts, clearly demonstrates that DCC, depleted of glycolipids and lipopolysaccharides, is a good antigenic preparation for evaluating T-cell reactivity to *M. leprae*.

Introduction

Although leprosy continues to be a major public health problem in many parts of the world,[1] WHO envisages that vigorous implementation of multidrug chemotherapy will decrease the prevalence of the disease to a negligible level by the year 2000;[2] also research to develop an antileprosy vaccine that will interrupt the transmission of the disease is

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being vigorously carried out.[3] Most individuals in leprosy-endemic areas develop a strong cellular immunity to *Mycobacterium leprae* and do not develop the disease,[3,4] and only a small minority become susceptible to the infection, and the reasons for their failure to develop a protective immunity against *M. leprae* are poorly understood.[5,6] An ideal antileprosy vaccine must be capable of inducing protective immunity against *M. leprae* in these individuals.[3] Prospective antileprosy vaccines, such as killed *M. leprae* and BCG, either alone or in combination, and 2 other closely-related mycobacteria, namely ICRC bacillus and *Mycobacterium w*, are currently undergoing field trials.[7] These vaccines were initially selected for their immunotherapeutic potential in leprosy patients before being used for clinical trials in apparently healthy individuals in a leprosy-endemic population.[3]

Many studies have demonstrated that *M. leprae* inhibits T-cell responses both *in vitro* and *in vivo*.[7–15] Certain cell surface components of *M. leprae*, such as phenolic glycolipid-I (PGL-I), lipoarabinomannan (LAM) and lipopolysaccharide, were shown to inhibit T lymphocyte proliferation[15–19] as well as interferon-γ (IFN-γ)-mediated activation of macrophages[20–22] *in vitro*. However, because it is not known how these immunomodulatory components of *M. leprae* in the vaccine preparations would influence the development of immunity to leprosy following vaccination, attempts have been made to remove these inhibitory substances from *M. leprae* to render it more immunogenic. Several studies have demonstrated that cell walls of *M. leprae* depleted of lipids, glycolipids and carbohydrate antigens induced strong T-cell proliferative responses,[23–27] induced IL-2 and IFN-γ synthesis,[28] augmented the killing of phagocytosed live *M. leprae* inside the macrophages,[29] elicited pronounced delayed type hypersensitivity reactions in sensitized guinea-pigs and tuberculoid leprosy patients,[27] and protected mice against leprosy bacilli.[30]

In this study, we evaluated the antigenicity of delipidified cell components of *M. leprae* (DCC, referred to previously as delipidified cell wall, DCW) by measuring the *in vitro* T lymphocyte reactivity to this in a large number of healthy contacts and leprosy patients across the disease spectrum, taken from a leprosy-endemic population in southern India. For comparison, the lymphoproliferative response to BCG and Dharmendra lepromin and the skin test reaction to the latter were also simultaneously measured.

Materials and methods

ANTIGENS

The Dharmendra preparation of *M. leprae* was generously supplied by Dr U. Sengupta, Central Jalma Institute for Leprosy, Agra, India. The BCG (Danish strain 1331) was kindly provided by The Director, BCG Vaccine Laboratories, Madras, India. It was subjected to Dharmendra treatment[31] and suspended in phosphate-buffered saline at a concentration of 10^7 bacilli per ml. Delipidified cell components of *M. leprae* (DCC) were prepared as described previously.[24] (Briefly, the pellet fraction of *M. leprae* sonicate extract was washed 3 times with chloroform: methanol (2:1). The residual material was further delipidified by extensive treatment with acetone and then with ethanol: ether (1:1). The final residue was suspended in saline and the protein concentration was determined.)

CELL CULTURE REAGENTS

Metrizoate sodium, Hanks's balanced salt solution, powdered culture medium, RPMI 1640 and penicillin-streptomycin mixture were purchased from Sigma Chemical Co., USA. Ficoll 400 was purchased from Pharmacia, Sweden.

BLOOD SAMPLES

Blood samples from leprosy patients, healthy family contacts (HFC) and healthy hospital contacts (HHC) were collected from a leposy hospital (Voluntary Health Services, Leprosy Project) located at Sakthinagar in the Periyar District, Tamil Nadu, India. The population covered by this leprosy control unit had a prevalence rate of 15·32 per 1000 at the beginning of this study (April 1989) and is covered by multidrug chemotherapy. Leprosy patients were classified clinically and bacteriologically[32] into polar lepromatous (LL), borderline lepromatous (BL), midborderline (BB), borderline tuberculoid (BT) and polar tuberculoid (TT) patients. HFC were healthy individuals living in the household of leprosy patients. Healthy hospital staff who had been exposed to leprosy patients for between 1 and 10 years were classified as HHC. We studied 162 samples, which had been collected from 96 leprosy patients (33 LL, 13 BL, 11 BB, 27 BT and 12 TT), 52 HFC and 14 HHC. Untreated and treated patients, under MDT for between 2 and 228 weeks, were included. LL and BL patients were grouped together and segregated into bacterial index (BI) positive (LBI+) and BI negative (LBI−) lepromatous patients. Patients with reactions were excluded from this study. We took 16 healthy noncontact (HNC) samples from students of the School of Biological Sciences, Madurai Kamaraj University, who had not had any habitual contact with leprosy patients even though they live in an endemic area. The study subjects were selected randomly without any bias towards age or sex. However, individuals below 12 years and above 70 years of age were not included. Each subject donated about 20 ml of venous blood into heparinized vacutainers (Vacuette; Griener, Germany).

LYMPHOPROLIFERATIVE ASSAYS

Peripheral blood mononuclear cells (PBMC), separated over a Ficoll-metrizoate density gradient,[33] were washed and suspended at a concentration of 1×10^6/ml in RPMI 1640 containing penicillin (100 U/ml), streptomycin (100 μg/ml) and 10% foetal calf serum (FCS) or normal human AB serum. Cultures with 10^5 cells in 200 μl final volume were stimulated with optimal concentration of PHA-P (10 μg/ml), Dharmendra lepromin (5×10^5 bacilli per ml), DCC (10 μg/ml) or BCG (5×10^5 bacilli/ml). The antigens are not cytotoxic at the concentrations used. Triplicate cultures in 96 well flat-bottom microtitre plates (Nunc, Denmark) were incubated at 37°C in a humidified atmosphere of 5% CO_2—95% air. Mitogen cultures were stimulated in FCS-containing medium for 3 days while antigen cultures were stimulated in human AB serum containing medium for 6 days. During the final 16 hr of the culture period, 0·5 μCi of ^{3}H-thymidine (Bhabha Atomic Research Center, Bombay, India, specific activity 6·7 Ci/mmol) was added to each well. Cultures were harvested onto glass fibre filters and the radioactivity incorporated was measured by a liquid scintillation counter (LKB Wallac, Sweden).

The results are expressed as ΔCPM (mean CPM of stimulated cultures − mean CPM

Table 1. *In vitro* lymphoproliferative responses to PHA-P, Dharmendra lepromin, delipidified cell components of *M. leprae* (DCC) and Dharmendra preparation of *M. bovis* BCG

Study groups	PHA-P	Dh. Lepromin	DCC	Dh. BCG
LBI+	31,028 ± 2,677† (51)‡	124 ± 129 (29)	3,149 ± 1,011* (33)	7,588 ± 1,335 (55)
LBI−	24,163 ± 2,167 (19)	275 ± 149 (8)	2,218 ± 1,008 (11)	10,981 ± 1,470 (19)
BB	25,426 ± 3,633 (13)	1,079 ± 712 (8)	4,258 ± 1,937 (11)	6,536 ± 2,233 (17)
BT	27,274 ± 2,570 (26)	1,938 ± 1,010 (7)	11,291 ± 4,911 (7)	13,908 ± 1,782 (36)
TT	21,722 ± 2,898 (16)	2,389 ± 1,168 (12)	6,843 ± 2,688 (5)	11,749 ± 2,424 (14)
HFC	32,208 ± 2,891 (53)	1,530 ± 1,024 (23)	8,499 ± 1,900* (52)	11,734 ± 1,660 (58)
HHC	36,825 ± 6,146 (16)	3,776 ± 3,627 (5)	14,192 ± 3,620* (14)	20,484 ± 5,171 (17)
HNC	40,825 ± 4,897 (14)	2,962 ± 1,583 (9)	13,197 ± 2,485* (16)	26,342 ± 5,478§ (15)

* Mean response significantly higher than that to Dharmendra lepromin within the group ($p < 0.05$).
† Mean ± SE of ΔCPM values.
‡ Number of individuals studied.
§ Mean response significantly higher than that of all other study groups ($p < 0.05$).

of control cultures) or stimulation index (SI = mean CPM of stimulated cultures/mean CPM of control cultures). Based on the range of response observed and the published literature, responses were considered positive when the SI was more than 3·0 and ΔCPM was more than 5000 for all antigens, and was more than 10,000 for PHA-P.

LEPROMIN SKIN TEST

A lepromin skin test was performed on the same day that blood samples were collected for lymphoproliferative assays. Indurations developed in response to intradermally inoculated Dharmendra lepromin (0·1 ml) were recorded 21 days postinoculation (late lepromin reaction).

STATISTICAL ANALYSIS

The Student's *t*-test and regression analyses were carried out using the EPISTAT statistical package.

Results

LYMPHOPROLIFERATIVE RESPONSE

PBMC from all groups of leprosy patients, healthy contacts and noncontacts showed poor *in vitro* proliferative response to Dharmendra lepromin (Table 1). None of the lepromatous patients responded to Dharmendra lepromin and, even in the other study groups only a small proportion of individuals showed any responsiveness (Figure 1). On the other hand, DCC induced a markedly higher proliferative response than Dharmendra lepromin in all the study groups (Table 1). Discernible gradation was observed in the proportion of responders to DCC, increasing from the lepromatous to tuberculoid pole (Figure 1). Such a gradation was not observed for the mitogenic response to PHA-P, which was uniformly high among the various study groups, including the lepromatous patient groups.

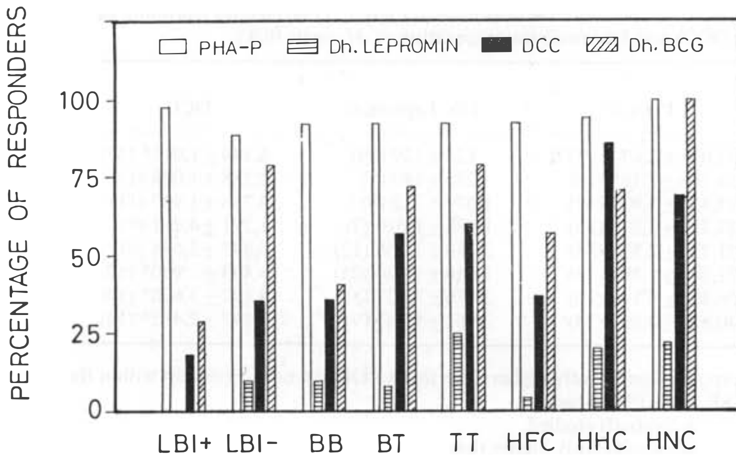

Figure 1. Proportion of responders to PHA-P, Dharmendra lepromin, DCC and Dharmendra preparation of BCG in *in vitro* lymphoproliferative assays. Responses were considered positive when SI was more than 3·0 and ΔCPM values were more than 10,000 for PHA-P and more than 5,000 for all antigens.

In contrast to Dharmendra lepromin, Dharmendra preparation of BCG induced good proliferative response in all groups of leprosy patients, contacts and noncontacts (Table 1). HNC showed significantly higher responses than all the other study groups. Interestingly, the proportion of BCG responders was significantly higher among LBI− patients than LBI+ patients (Figure 1). It should be noted that responses to DCC and BCG were markedly low among HFC compared to HHC, while their responses to PHA-P were comparable (Table 1).

Analysis of the pooled data from all study groups revealed a significant positive correlation between the responses to DCC and BCG (Figure 2). Almost all responders to DCC responded to BCG as well (45/51). About 40% of the subjects did not respond to both DCC and BCG, and the majority of these individuals belonged to lepromatous and BB patients (Table 2). A considerable proportion of HFC also failed to respond to both. In contrast, the majority of the HHC responded to both the antigens. Strikingly, 23 out of

Table 2. Comparison of the lymphoproliferative responses to BCG and DCC in leprosy patients, healthy contacts and non-contacts

		Percentage of			
Study groups	N*	BCG R DCC R	BCG R DCC NR	BCG NR DCC R	BCG NR DCC NR
LBI+	28	18	7	4	71
LBI−	3	33	67	0	0
BB	9	22	22	11	45
BT/TT	9	56	22	0	22
HFC	47	30	23	2	45
HHC	14	64	14	22	0
HNC	11	82	18	0	0

* N = number of individuals studied in each group. Responders (R) and non-responders (NR) are defined in Figure 1.

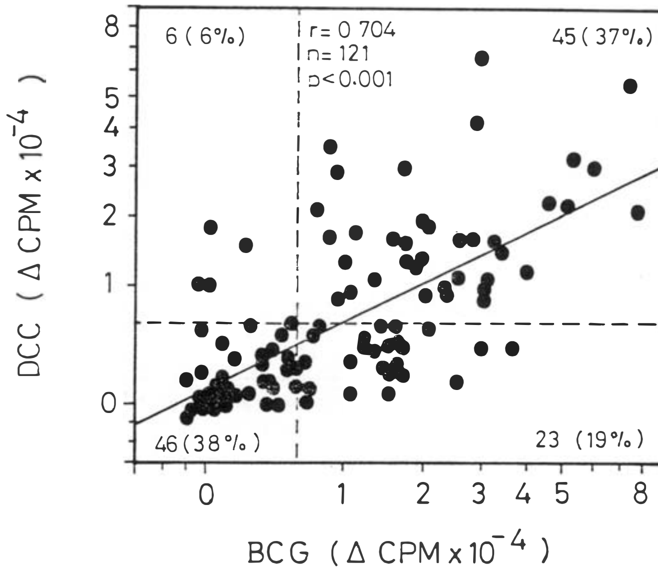

Figure 2. Correlation between the responses to DCC and BCG. Data from all leprosy patient groups, healthy contacts and noncontacts were pooled and the correlation between the responses to DCC and BCG was evaluated by regression analysis. Vertical and horizontal dashed lines bisecting the x and y axes represent the cutoff values for positive response to BCG and DCC, respectively (for details see Fig. 1). For each plot, the number and the percentage (in parentheses) of individuals within each quadrant are mentioned. r, correlation coefficient; n, total number of subjects studied; p, significance level of the correlation.

Table 3. Skin test response to Dharmendra lepromin in leprosy patients and healthy contacts

		Late lepromin reaction						
Study groups	(N)†	Diameter (mm): Score	0 (−)	<3 (±)	3–5 (+)	6–10 (++)	>10 or Ulcer (+++)	Percentage of responders*
LBI+	(41)		41	—	—	—	—	0
LBI−	(10)		10	—	—	—	—	0
BB	(13)		11	1	—	1	—	8
BT	(20)		8	—	1	7	4	60
TT	(17)		2	—	2	9	4	88
HFC	(60)		20	1	16	17	6	65
HHC	(12)		5	—	1	4	2	58

We inoculated 0·1 ml of Dharmendra lepromin intradermally in the forearm. The induration was measured after 21 days. Based on the diameter of induration, scores are defined arbitrarily. Skin testing was not done on healthy noncontacts.

† (N) = number of individuals tested in each group. Values given are number of individuals in each category.

* Responders are defined as individuals who developed indurations measuring >3 mm in diameter.

68 BCG responders did not respond to DCC (Figure 2) and these subjects were represented in all groups of leprosy patients, HFC, HHC and HNC (Table 2).

IN VIVO SKIN TEST REACTION TO DHARMENDRA LEPROMIN

Despite its failure to induce *in vitro* lymphoproliferative response uniformly in all groups of leprosy patients and healthy controls, Dharmendra lepromin elicited a marked late lepromin reaction in tuberculoid patients and healthy contacts (Table 3). Lepromatous

Table 4. Comparison of the *in vivo* response to Dharmendra lepromin and *in vitro* response to DCC

Skin test response to Dharmendra lepromin:		R	R	NR	NR
Lymphoproliferative response to DCC:		R	NR	R	NR
Study groups	(N)*		(percentage)		
LBI+	(33)	0·00	0·00	18·18	81·82
LBI−	(5)	0·00	0·00	12·50	87·50
BB	(11)	0·00	9·09	36·36	54·55
BT	(7)	28·57	14·29	28·57	28·57
TT	(5)	60·00	40·00	0·00	0·00
HFC	(50)	22·00	40·00	16·00	22·00
HHC	(12)	41·67	16·67	41·67	0·00

* N = Number of individuals tested in each group. Responders (R) and non-responders (NR) are defined in Table 3 and Figure 1.

patients, irrespective of their BI status, and BB patients did not develop a skin test reaction to Dharmendra lepromin.

Analysis of the data revealed that in a considerable proportion of individuals among all study groups (except TT) who failed to mount lepromin reaction *in vivo*, DCC elicited a positive lymphoproliferative response (Table 4). Conversely, among tuberculoid patients and healthy contacts a significant proportion of individuals did not respond to DCC *in vitro*, but developed a lepromin reaction *in vivo*.

Discussion

Various preparations of *M. leprae*, such as whole bacilli, Dharmendra lepromin, sonicate extracts and cell wall components have been used in *in vitro* assays to measure T lymphocyte reactivity to the antigens of *M. leprae* in leprosy patients and healthy contacts.[9,34–38] Recent investigations have demonstrated that PGL-I and LAM which accumulate on the surface of *M. leprae* are the major immunomodulatory components capable of inhibiting both T cell proliferation[16–19] and macrophage activation and effector functions.[20,21] In fact, LAM has been shown to inhibit the transcription of IL-2 gene in T lymphocytes[39] and IFN-γ inducible genes in mononuclear phagocytes.[40] Most individuals exposed to leprosy bacilli in endemic areas overcome the adverse effects of these components and develop strong cellular immune responses and protective immunity to the pathogen.

In the present study involving a large number of leprosy patients and healthy contacts, we observed that Dharmendra lepromin induced poor *in vitro* lymphoproliferation in all study groups though others have shown reactivity in tuberculoid patients and healthy contacts.[9,34] In contrast, Dharmendra preparation of BCG induced proliferation of PBMC from all groups including the BI negative lepromatous patients, indicating that Dharmendra treatment[31] of mycobacteria by chloroform and ether *per se* does not affect the antigenic constituents. The fact that Dharmendra lepromin induced marked skin test reactions in tuberculoid patients and healthy contacts argues against any loss of antigenic material in the preparation, as well as against the lack of immunological reactivity in the

study subjects towards *M. leprae*. Earlier studies from our laboratory have demonstrated that Dharmendra lepromin inhibited T cell proliferative responses of normal subjects to mitogens and antigens, and this was associated with the downregulation of CD2 expression on T lymphocyte surface.[13,14] However, DCC did not modulate CD2.[41] Presumably, the immunomodulatory components of *M. leprae* present in Dharmendra lepromin would obscure the stimulatory effect of its antigenic constituents. In contrast to Dharmendra lepromin, whole bacilli have been shown to elicit a T cell proliferative response in tuberculoid patients.[35] Therefore, it is likely that the immunomodulatory components are more exposed on Dharmendra lepromin than whole bacilli by limited treatment with organic solvents. However, induction of marked skin test reactions by Dharmendra lepromin suggests that the immunomodulatory components are either diluted out or degraded *in vivo*, and the antigenic components released slowly from the intact bacilli elicit a strong late lepromin reaction.

DCC induced a significantly higher level of lymphoproliferative response than Dharmendra lepromin in all groups of leprosy patients, healthy contacts and non-contacts. A significant positive correlation observed between the responses to DCC and BCG reflects the close antigenic similarity between *M. leprae* and *M. bovis* BCG. However, about 20% of the subjects belonging to all study groups responded to BCG but not to DCC, indicating that responsiveness to BCG is not always associated with a positive response to DCC. This is probably due to the qualitative and quantitative differences in the antigenic composition of DCC and BCG, as much as the variability in the immune response of an individual.

Our results show that DCC is indeed a better antigenic preparation than Dharmendra lepromin for measuring the T lymphocyte response to *M. leprae*. In fact, DCC induced a lymphoproliferative response even in those individuals who failed to show an *in vivo* skin test reaction to Dharmendra lepromin. On the other hand, a positive skin-test response to Dharmendra lepromin in the absence of demonstrable T cell reactivity to DCC *in vitro* could be explained by a very low frequency of T lymphocytes reactive to *M. leprae* in the peripheral circulation which could have been recruited to the inoculation site over the period of 21 days. It has also been demonstrated that purified cell wall preparations of *M. leprae* induced more pronounced skin test reactions than lepromin in tuberculoid leprosy patients.[27] Depletion of lipids and carbohydrates has rendered DCC more antigenic, presumably by relieving the immunomodulatory effects on antigen-presenting macrophages and effector T lymphocytes. Though the immunomodulatory components present in whole *M. leprae* preparations used for vaccination are likely to be cleared by catabolic processes, they can delay the induction of specific immunity, especially at doses given for vaccination compared to that used for skin testing. Therefore, until the immunogenic constituents of *M. leprae* are defined at molecular level, delipidified antigenic preparations of *M. leprae* would be ideal alternatives to whole bacilli in leprosy vaccination strategies.

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References

[1] Noordeen SK. A look at world leprosy. *Lepr Rev*, 1991; **62:** 72–86.

[2] Noordeen SK. Elimination of leprosy as a public health problem. *Lepr Rev*, 1992; **63:** 1–4.

[3] Watson JD. Prospects for new generation vaccines for leprosy: progress, barriers and future strategies. *Int J Lepr*, 1989; **57:** 834–43.

[4] Bloom BR. Learning from leprosy: a perspective on immunology and the third world. *J Immunol*, 1986; **137:** i–x.

[5] Bach MA, Lanouis P. Mechanisms of *Mycobacterium leprae*-specific T-cell deficiency in lepromatous leprosy. *Biochimie* 1988; **70:** 1013–18.

[6] Kaplan G, Cohn ZA. Leprosy and cell mediated immunity. *Current Opinion in Immunol*, 1991; **3:** 91–6.

[7] Gupte MD. Vaccines against leprosy. *Ind J Lepr*, 1991; **63:** 342–49.

[8] Bjune G. *In vitro* lymphocyte stimulation in leprosy; simultaneous stimulation with *Mycobacterium leprae* antigens and phytohaemagglutinin. *Clin exp Immunol*, 1979; **36:** 479–87.

[9] Mehra V, Mason L, Fields JP, Bloom BR. Lepromin-induced suppressor cells in patients with leprosy. *J Immunol*, 1979; **123:** 1813–17.

[10] Touw J, Stoner GL, Belehu A. Effect of *Mycobacterium leprae* on lymphocyte prolfieration: suppression of mitogen and antigen responses of human peripheral blood mononuclear cells. *Clin exp Immunol*, 1980; **41:** 397–405.

[11] Nath I, Singh R. The suppressive effect of *M. leprae* on the *in vitro* proliferative responses of lymphocytes from patients with leprosy. *Clin exp Immunol*, 1980; **41:** 406–14.

[12] Bahr GM, Rook GAW, Stanford JL. Inhibition of the proliferative response of peripheral blood lymphocytes to mycobacterial or fungal antigens by co-stimulation with antigens from various mycobacterial species. *Immunology*, 1981; **44:** 593–98.

[13] Muthukkaruppan VR, Chakkalath HR, James MM. Immunologic unresponsiveness in leprosy is mediated by modulation of E-receptor. *Immunol Letters*, 1987; **15:** 199–204.

[14] Muthukkaruppan VR, Chakkalath HR, Malarkannan S. The classical and alternate pathways of T cell activation are impaired in leprosy. *Immunol Letters*, 1988; **19:** 55–58.

[15] Sengupta U, Sinha S, Ramu G, Lamb J, Ivanyi J. Suppression of delayed hypersensitivity skin reactions to tuberculin by *M. leprae* antigens in patients with lepromatous and tuberculoid leprosy. *Clin exp Immunol*, 1987; **68:** 58–64.

[16] Mehra V, Brennan PJ, Rada E, Convit J, Bloom BR. Lymphocyte suppression in leprosy induced by unique *Mycobacterium leprae* glycolipid. *Nature*, 1984; **308:** 194–96.

[17] Prasad HK, Mishra RS, Nath I. Phenolic glycolipid-I of *Mycobacterium leprae* induces general suppression of *in vitro* concanavalin A responses unrelated to leprosy type. *J Exp Med*, 1987; **165:** 239–45.

[18] Kaplan G, Gandhi RR, Weinstein DE, Levis WR, Patarroyo ME, Brennan PJ, Cohn ZA. *Mycobacterium leprae* antigen-induced suppression of T cell proliferation *in vitro*. *J Immunol*, 1987; **138:** 3028–34.

[19] Molloy A, Gaudernack G, Levis WR, Cohn ZA, Kaplan G. Suppression of T cell proliferation by *Mycobacterium leprae* and its products. The role of lipopolysaccharide. *Proc Natl Acad Sci USA*, 1990; **87:** 973–77.

[20] Sibley LD, Hunter SW, Brennan PJ, Krahenbhul JL. Mycobacterial lipoarabinomannan inhibits gamma interferon mediated activation of macrophages. *Infect Immun*, 1988; **56:** 1232–36.

[21] Vachula M, Holzer TJ, Andersen BR. Suppression of monocyte oxidative response by phenolic glycolipid I of *Mycobacterium leprae*. *J Immunol*, 1989; **142:** 1696–1701.

[22] Kaplan G, Cohn ZA. The immunobiology of leprosy. *Int Rev Exp Pathol*, 1986; **28:** 45–78.

[23] Khandke L, Salgame PR, Mahadevan PR. *In vitro* lymphocyte stimulating ability of cell components of *Mycobacterium leprae*. IRCS Med Sci, 1984; **12:** 1012–13.

[24] Robinson P, Mahadevan PR. A component of *Mycobacterium leprae* as immunomodulating agent for immune deficient cells of leprosy patients. *J Clin Lab Immunol*, 1987; **24:** 171–6.

[25] Hunter SW, McNeil M, Modlin RL, Mehra V, Bloom BR, Brennan PJ. Isolation and characterization of highly immunogenic cell wall-associated protein of *Mycobacterium leprae*. *J Immunol*, 1989; **142:** 2864–72.

[26] Mehra V, Bloom BR, Torigian VK, Mandich D, Reichel M, Young SMM, Salgame P, Convit J, Hunter SW, McNeil M, Brennan PJ, Rea TH, Modlin RL. Characterization of *Mycobacterium leprae* cell wall associated proteins with the use of T lymphocyte clones. *J Immunol*, 1989; **142:** 2873–78.

[27] Meloncoln-Kaplan J, Hunter SW, McNeil M, Stewart C, Modlin RL, Rea TH, Convit J, Salgame P, Mehra V, Bloom BR, Brennan PJ. Immunological significance of *Mycobacterium leprae* cell walls. *Proc Natl Acad Sci USA*, 1988; **85:** 1917–21.

[28] Mahadevan PR, Robinson P. An antigenic complex that restores ability in leprosy patients to kill *Mycobacterium leprae*—the probable molecular events identified by *invitro* experiments. *Trop Med Parasitol* 1990; **41:** 310–13.

[29] Morolia J, Robinson P, Mahadevan PR. A complete component modulating immune-deficient cells in leprosy patients leading to loss of viability of *Mycobacterium leprae*—a possible vaccine. *Clin exp Immunol*, 1990; **79:** 7–14.

[30] Gelber RH, Brennan PJ, Hunter SW, Munn MW, Monson JM, Murray LP, Siu P, Tsang M, Engleman EG, Mohagheghpour N. Effective vaccination of mice against leprosy bacilli with subunits of *Mycobacterium leprae*. *Infect Immun*, 1990; **58:** 711–8.
[31] Sengupta U, Ramu G, Desikan KV. Assessment of Dharmendra antigen II. Standardisation of the antigen. *Lepr India* 1979; **51:** 316–322.
[32] Ridley DS, Jopling WH. Classification of leprosy according to immunity: a five group system. *Int J Lepr*, 1966; **34:** 255–73.
[33] Hunt SV. Preparative immunoselection of lymphocyte populations. In: Weir DM. (ed) *Handbook of Experimental Immunology*, 4th edn. London: Blackwell Scientific Publications, 1986; 55.11.
[34] Bjune G. Variation of *in vitro* lymphocyte responses to *M. leprae* antigen in borderline tuberculoid leprosy patients. *Int J Lepr*, 1980; **48:** 30–40.
[35] Bach MA, Wallac D, Flaguel B, Cottenot F. *In vitro* response to *M. leprae* and PPD of isolated T cell subsets from leprosy patients. *Clin exp Immunol*, 1983; **52:** 107–14.
[36] Gill HK, Mustafa AS, Godal T. Vaccination of human volunteers with heat-killed *M. leprae*: local responses in relation to the interpretation of the lepromin reaction. *Int J Lepr*, 1988; **56:** 36–44.
[37] Mullins R, Basten A. Effect of preincubation on the proliferative response to antigen by cells from leprosy patients and healthy controls. *Int J Lepr*, 1989; **57:** 777–87.
[38] Closs O, Reitan LJ, Negassi K, Harboe M, Belehu A. *In vitro* stimulation of lymphocytes in leprosy patients, healthy contacts of leprosy patients, and subjects not exposed to leprosy. Comparison of an antigen fraction prepared from *Mycobacterium leprae* and tuberculin purified protein derivative. *Scand J Immunol*, 1982; **16:** 103–15.
[39] Chujor CSN, Ruhn B, Schwerer B, Bernheimer H, Levis WR, Bevec D. Specific inhibition of mRNA accumulation for lymphokines in human T cell line Jurkat by mycobacterial lipoarabinimannan antigen. *Clin exp Immunol*, 1992; **87:** 398–403.
[40] Chan J, Fan X, Hunter SW, Brennan PJ, Bloom BR. Lipoarabinomannan, a possible virulence factor involved in the presence of *Mycobacterium tuberculosis* within macrophages. *Infect Immun*, 1991; **59:** 1755–61.
[41] Sheela R, Ilangumaran S, Muthukkaruppan VR. Flow cytometric analysis of CD2 modulation on human peripheral blood lymphocytes by Dharmendra preparation of *Mycobacterium leprae*. *Scand J Immunol*, 1991; **33:** 203–9.

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Réactivité aux constituants cellulaires délipidifiés de *Mycobacterium leprae* des T-lymphocytes de malades lépreux et de contacts sains appartenant à une population où la lèpre est endémique

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Résumé Nous avons mesuré la prolifération *in vitro* des mononucléaires du sang périphérique en réponse à la présence des constituants cellulaires délipidifiés de *Mycobacterium leprae* (DCC) et de la lépromine Dharmendra chez, d'une part, des lépreux choisis dans tout le spectre clinique et, d'autre part, des contacts sains pris dans une population où la lèpre est endémique. La lépromine Dharmendra a provoqué une médiocre prolifération des cellules T *in vitro* dans tous les groupes de l'étude, bien qu'elle ait suscité *in vivo* une réaction nette au test cutané dans les cas de lèpre tuberculoïde et chez les contacts sains. Par contre, la préparation Dharmendra de BCG a provoqué une réponse nette des cellules T tant chez les malades lépromateux tuberculoïdes que chez ceux à indice bactériel négatif. DCC a provoqué une lymphoprolifération significativement plus élévée que la lépromine Dharmendra chez tous les groupes de l'étude. Nous avons observé une corrélation positive significative entre les réponses à DCC et BCG. Cette étude, basée sur un grand nombre de malades lépreux et de contacts sains démontre clairement que DCC, après élimination des glycolipides et des lipopolysaccharides, constitue une bonne préparation antigénique pour évaluer la réactivité des cellules T à *M. leprae*.

La reactividad de T Linfocitos de los pacientes leprosos y contactos sanos de poblaciones con lepra endémica, a los componentes de células delipidificadas de *Mycobacterium leprae*

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Resumen En este estudio, se midieron las respuestas proliferatives de las células mononucleares hemáticas periféricas tanto de los pacientes leprosos con una extensa gama clínica, como de los contactos sanos de poblaciones con lepra endémica, a los componentes de células delipidificadas de *Mycobacterium leprae* (DCC) y Dharmendra lepromin. El Dharmendra lepromin tenía poca efectividad para inducir proliferación de células T in vitro en todos los grupos estudiados, aunque provocó una reacción dérmica in vivo en los leprosos tuberculoides y en los contactos sanos. En cambio, la preparación Dharmendra de BCG indució una respuesta definitiva de células T en los pacientes tuberculosos además de en los pacientes lepromatosos con índice bacteriano negativo. DCC indució una respuesta lainfoproliferativa significativamente mayor que el Dharmendra lepromin en todos los grupos estudiados. Se observó una correlación positiva significativa entre las respuestas linfoprolifertivas al DCC y el BCG. Este estudio, basado en un gran número de leprosos y contactos sanos, demuestra claramente que DCC, con reducido glicolípidos y lipopolisacaridos, es una buena preparación antigénica para la evaluación de la reactividad de células T a *M. leprae*.