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The effect of *Mycobacterium leprae* on PHA- and PPD-induced inhibition of leucocyte migration in leprosy patients

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Summary The effect of *Mycobacterium leprae* was studied on mitogen, PHA and antigen, PPD-induced leucocyte migration inhibition in 44 leprosy patients and 13 healthy controls using the leucocyte migration inhibition test. While *M. leprae* decreased the PHA-generated inhibition of migration of leucocytes in tuberculoid patients and healthy individuals, it enhanced the inhibitory effect on the leucocyte migration in lepromatous patients. However, a uniform decrease by *M. leprae* was observed on PPD-induced leucocyte migration inhibition in both groups of leprosy patients and healthy controls.

Introduction

The nature of resistance in tuberculoid (TT) leprosy and lack of it in lepromatous leprosy (LL) remains unknown. Bacillary load in the former is absent or minimal while in the latter it is high. The anergy obtained in LL may be attributed to this. The effect of *Mycobacterium leprae* on the blastogenic responses of peripheral blood mononuclear cells of leprosy patients induced by mitogens, Phytohaemagglutinin (PHA) and Concanavalin-A (Con-A) and a cross-reacting antigen, purified protein derivative (PPD) of tuberculin has been reported by some investigators.[1,7,13] Suppression or enhancement of the blastogenic responses not only varied with the type of leprosy but also was dependent on the *M. leprae* preparation, whole or sonicated. Two studies,[5,7] came to opposite conclusions using Dharmendra antigen and whole autoclaved *M. leprae* respectively on the inducibility of *M. leprae* specific suppression in leprosy polar groups. We report here observations on the effect of *M. leprae* on PHA- and PPD-induced release of lymphokine effecting leucocyte migration in the leucocyte migration inhibition test (LMIT).

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Materials and methods

TEST SUBJECTS

Forty-four leprosy patients, 18 LL and 26 TT attending out-patient departments of Gandhi Hospital, Sivananda Rehabilitation Home and Dhoolpet Leprosy Research Centre, all in Hyderabad City, South India, were included in the study. The lepromatous group here, includes 15 polar lepromatous type (LL) and 3 borderline lepromatous (BL) patients and the tuberculoid group includes 10 polar tuberculoid (TT) and 16 borderline tuberculoid (BT) patients. All of them were untreated. They were classified on the Ridley–Jopling scale.[11] The control group comprised of 13 healthy contacts who have been working in the field of leprosy for 3 to 5 years.

MITOGEN AND ANTIGEN

Phytohaemagglutinin-P (PHA) was a Difco product and purified protein derivative (PPD) of tuberculin (Mammalian) was obtained from Staten Serum Institut, Copenhagen. 1 μl/ml of PHA and 25 μg/ml of PPD were found to be optimal in our LMIT system (data not shown), and were used as optimal dose in this study. Armadillo-derived whole *M. leprae*, Batch No. AB 51, supplied by National Institute of Medical Research, London, was used at a concentration of $2{\cdot}5 \times 10^7$ bacilli/ml which was shown as optimal dose in LMIT in an earlier study from our laboratory.[4]

LEUCOCYTE MIGRATION INHIBITION TEST

The original method[12] was used with some modifications. Briefly to 15 ml of acid citrate dextrose (ACD) anti-coagulated blood, 7·5 ml of 3% gelatin (Sigma Chemical Co., USA) in saline was added in a culture tube. After thorough mixing, it was incubated for 45 min at a slanting position at 37°C to sediment erythrocytes and leucocyte-rich plasma was aspirated. After centrifugation, pelleted leucocytes were washed with Minimum Essential Medium (MEM), Bios, Bombay and finally resuspended in MEM to give a concentration of 30×10^6 cells/ml. Viability was checked with 0·2% Trypan Blue. The cell suspension was loaded into capillaries (Arthur Thomas Co., USA), sealed with modelling clay at one end and centrifuged at 1000 rpm for 5 min. The capillaries were then cut at the cells-medium interface and were kept in polystyrene chambers (Laxbro, Pune). Immediately, chambers were filled with MEM containing 20% foetal calf serum (FCS), Microlab, Bombay with or without antigen or mitogen and then sealed with cover-slips and incubated at 37°C for 18 h. Each test was run in triplicate. The areas of migration were measured with a planimeter. The migratory index (MI) was calculated as follows:

$$MI = \frac{\text{Average area of migration with antigen or mitogen}}{\text{Average area of migration with medium alone}}.$$

$$\text{Migratory Inhibition} = 1 - MI$$

Effect of *M. leprae* on PHA- and PPD-induced effect on leucocyte migration was observed with addition of *M. leprae* simultaneously with PHA or PPD by noting the percentage change in average area of migration with the following formula.

% *M. leprae* effect on PHA or PPD:

$$100 - \frac{(\%\text{ Migratory inhibition of } M.\ leprae + \text{PHA or PPD})}{\left(\begin{matrix}\%\text{ Migratory inhibition} \\ \text{of } M.\ leprae \text{ alone}\end{matrix}\right) + \left(\begin{matrix}\%\text{ Migratory inhibition} \\ \text{of PHA or PPD alone}\end{matrix}\right)} \times 100.$$

The increased/decreased area of migration of leucocytes in *M. leprae*- PHA or PPD combination tests, compared to the area of migration in PHA or PPD alone tests, were shown as *M. leprae* induced inhibition (−) enhancement (+) response to PHA or PPD respectively.

Two sampled Students 't' test and Median test were used for statistical analysis.[2]

Results

No significant differences were observed in the mean Migratory Index (MI) values of PHA and PPD among the 2 leprosy patient groups and healthy control group. The mean MI value of *M. leprae* of the LL group (0·97) showed a highly-significant difference ($P < 0{\cdot}001$) when compared to that of the TT patient group (0·81) and healthy control group (0·85) (Table 1 and Table 2). There was no correlation between the responses of *M. leprae* and PPD in all the 3 groups of subjects studied (lepromatous group r = 0·0102; tuberculoid group r = 0·1560; healthy control group r = 0·3826).

EFFECT OF *M. LEPRAE* ON PHA

Table 1 and Figure 1 give the details of statistics and pattern of effect of *M. leprae* on PHA responses respectively. Thirteen out of 18 LL group patients showed enhanced PHA responses on addition of *M. leprae* whereas 5 showed inhibition, with an overall median percent of enhancement of +6·0. Out of the 26 TT group patients, 19 showed inhibitory responses whereas 7 showed enhanced responses with an overall median percent of inhibition of −40·0. Eleven out of 13 healthy contacts showed inhibitory responses and only 2 showed enhanced responses with an overall median percent of inhibition of −39·0. Inhibition of PHA

Table 1. Effect of whole *M. leprae* on PHA-induced inhibition of leucocyte migration in the comparing groups

	Migratory index Mean ± SE (median)		
	Lepromatous group	Tuberculoid group	Healthy control group
Whole *M. leprae*	0·97 ± 0·01 (0·99)	0·81 ± 0·02* (0·81)	0·85 ± 0·02* (0·84)
PHA	0·57 ± 0·05 (0·55)	0·51 ± 0·03 (0·52)	0·59 ± 0·03 (0·62)
Whole *M. leprae* + PHA	0·50 ± 0·05 (0·43)	0·58 ± 0·03 (0·61)	0·65 ± 0·06 (0·69)
Median % effect of *M. leprae* on PHA	+6·0	−40·0†	−39·0†
Number of subjects	18	26	13

−, Indicates Inhibition; +, indicates Enhancement.

* Indicates $P < 0·001$ significance by Students 't' test compared to Lepromatous group.

† Indicates $P < 0·01$ significance by Median test compared to lepromatous group.

Table 2. Effect of whole *M. leprae* on PPD-induced inhibition of leucocyte migration in the comparing groups

	Migratory index Mean ± SE (median)		
	Lepromatous group	Tuberculoid group	Healthy control group
Whole *M. leprae*	0·97 ± 0·01 (0·99)	0·81 ± 0·02* (0·81)	0·85 ± 0·02* (0·84)
PPD	0·76 ± 0·06 (0·68)	0·74 ± 0·05 (0·75)	0·63 ± 0·06 (0·65)
Whole *M. leprae* + PPD	0·79 ± 0·05 (0·71)	0·74 ± 0·05 (0·77)	0·66 ± 0·05 (0·68)
Median % effect of *M. leprae* on PPD	−25·0	−41·0	−43·0
Number of subjects	18	21	13

− Indicates Inhibition.

* Indicates $P < 0·001$ significance by Students 't' test compared to lepromatous group.

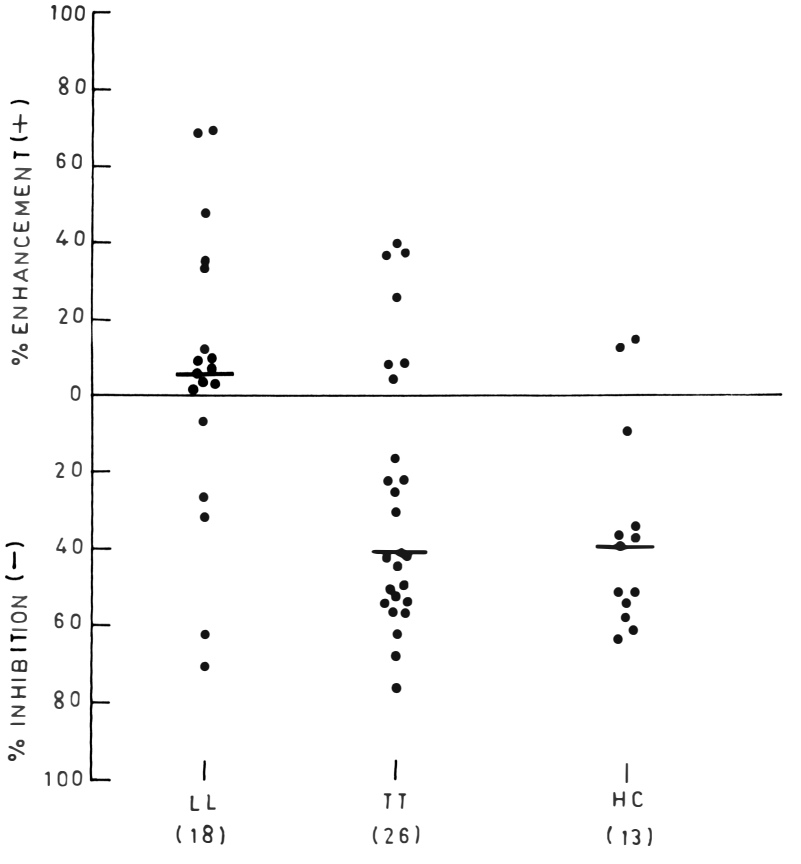

Figure 1. Effect of *M. leprae* on individual PHA responses in lepromatous (LL), tuberculoid (TT) and healthy contact (HC) groups (− denotes median).

responses in the TT and healthy contact groups was very significant ($P<0{\cdot}01$) when compared to the LL group.

EFFECT OF *M. LEPRAE* ON PPD RESPONSES

The effect of *M. leprae* on PPD responses are given in Table 2 and pattern in Figure 2, for the comparing categories respectively. Thirteen out of the 18 LL group patients showed inhibitory responses to PPD on addition of *M. leprae* and the remaining showed enhanced responses with an overall median percent inhibition of −25·0. Out of the 21 TT group, 16 showed inhibitory responses whereas 5 showed enhanced responses, with an overall median percent inhibition of −41·0. Among the healthy contacts, 10 showed inhibitory responses whereas 3 showed enhanced responses, with an overall median percent inhibition of −43·0. There is no significant difference between the 3 groups compared.

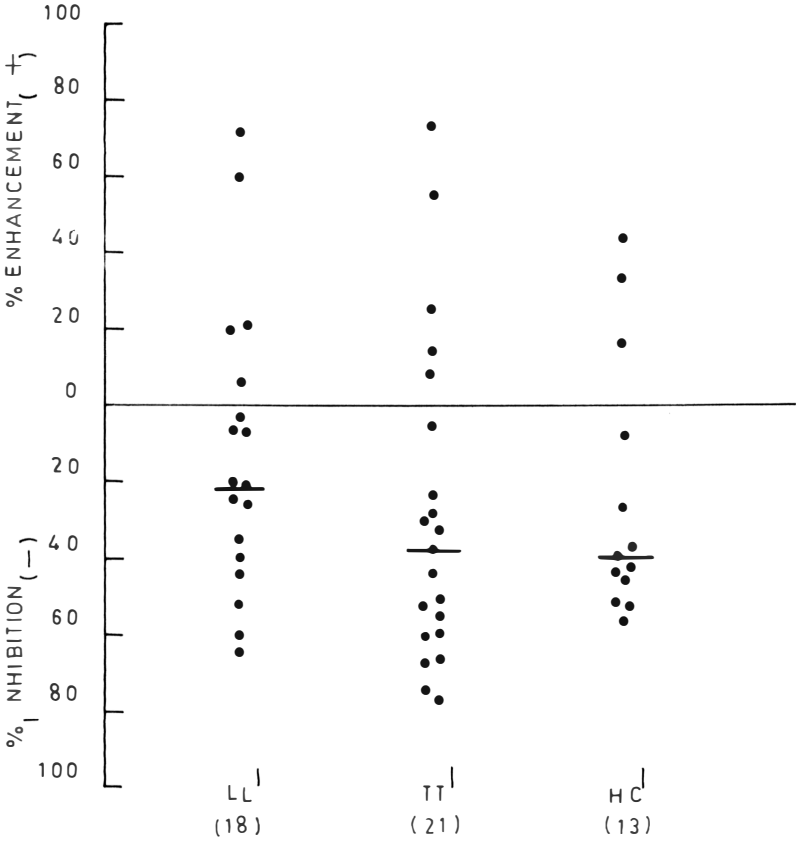

Figure 2. Effect of *M. leprae* on individual PPD responses in lepromatous (LL), tuberculoid (TT) and healthy contact (HC) groups (− denotes median).

Discussion

It has been reported[1] that sonicated *M. leprae* suppressed PHA-induced blastogenic responses of peripheral blood mononuclear cells of all leprosy patients and contact individuals. Under similar conditions, whole *M. leprae* showed stimulatory proliferative responses to BT patients but had no effect on BL and LL patients. Similarly, it has been reported[13] that the *in vitro* blastogenic response of PHA stimulated peripheral blood mononuclear cells can be suppressed by the addition of *M. leprae* among LL, TT patients and healthy volunteers. No significant suppressive effect was observed with PPD. However, one study[7] has reported uniform suppression with PPD in all leprosy patients.

Some elegant studies on the antigen specific suppressive effects of *M. leprae* utilized Con-A as a mitogen and inducer of suppressor cells for blastogenic responses of the peripheral blood mononuclear cells *in vitro* from leprosy patients. One study[5, 6] has reported inhibition of blastogenic responses by

Dharmendra lepromin in the LL and BL patients and further showed that TH_2^+/OKT8$^+$ cells associated with suppressor activity are involved. On the other hand, another study[7] using whole, autoclaved *M. leprae* showed suppression of Con-A induced lymphocyte proliferation in TT patients, whereas there was enhancement in LL patients. It was also shown that there is an absence of hyperactive clones of suppressor cells in LL patients.[8]

Recent studies, however, have shown that LL patients have defective lymphocytes and that the lack of response is not due to absence of *M. leprae* reactive T cells. One study[3] has shown that addition of Interleukin-2 (IL-2) to peripheral blood mononuclear cells from LL patients makes them respond to proliferative stimulus of *M. leprae*. Further, another study[9] shows that peripheral blood mononuclear cells from LL patients are defective in the production of gamma Interferon essential for activation of macrophages and this defect was partially restored by the addition of purified IL-2 and *M. leprae* antigen to the culture medium and not with IL-2 alone.

We have measured the effect of *M. leprae* on a different parameter, the migration of leucocytes which is inhibited by a lymphokine, leucocyte inhibitory factor (LIF) released by peripheral blood mononuclear cells when they are stimulated with a mitogen or an antigen. PHA, a mitogen, inhibited the migration of leucocytes in normal individuals and leprosy patients to the same extent (Table 1). *M. leprae* antigen also slows down the migration of leucocytes in TT patients which is of the same magnitude as in normal contacts. However, LL patients do not give this response. When *M. leprae* was added along with PHA in this study, the inhibitory response was more than additive in LL patients whereas it was reduced in TT patients. The less than additive effect in TT patients is as expected when 2 simultaneous stimuli are applied. However, the enhanced response in LL patients is interesting. PHA is a potent inducer of IL-2 secretion and preferentially stimulates OKT4$^+$, a helper subset of T lymphocytes.[10] The synergic effect observed when PHA and *M. leprae* are added together, is due to the release of IL-2 by PHA which in the presence of *M. leprae* brings out the enhanced response in LL cells and which *M. leprae* alone cannot elicit. These results are identical to the observations[3] which demonstrated proliferative responses of peripheral blood mononuclear cells after addition of IL-2 along with *M. leprae* and indicate that *M. leprae* reactive T cells are present in the LL patients and IL-2 makes them respond to *M. leprae* stimulus. It would be interesting to observe if IL-2 replaces PHA in its effect on leucocyte migration of LL patients.

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