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Suppression of *Mycobacterium leprae*-induced leucocyte migration inhibition following lepromin injection in healthy contacts of leprosy. Preliminary observations

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Summary Lymphokine production to PHA and *Mycobacterium leprae* was measured using the leucocyte migration inhibition test before and after lepromin skin testing in 7 healthy contacts of leprosy patients. There was suppression of responses to *M. leprae* following lepromin injection, but the responses to PHA were unaffected: this may indicate the presence of protective immunity to leprosy in these subjects.

Introduction

Leprosy is a very chronic disease. Infectious cases are likely to excrete *M. leprae* for months or years prior to diagnosis, and for weeks or months after the commencement of treatment, thus exposing their household contacts to prolonged bombardment with antigenic material. The normal response of exposed subjects is the development of protective immunity; few acquire progressive disease. However, the prolonged exposure suggests a special need for a mechanism to avoid the development of an overactive immune response which could be harmful to the subject.

A possibly suitable control mechanism has been demonstrated *in vitro*. Regulation of the immune response is a function of suppressor cells, and mycobacterial antigens have been shown to induce suppressor cells which exerted antigen-specific suppression in lymphocyte cultures. The subjects were healthy

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individuals who were probably immunized against leprosy by prolonged exposure[11] or against tuberculosis by BCG vaccination.[4] Similarly another study[9] demonstrates *M. leprae* induced suppression of Con A responses of mononuclear cells from armadillos which appeared to be resistant to infection with *M. leprae*. This rather consistent pattern of results contrasts with the highly varied results of similar studies in which cell cultures from leprosy patients were used.[1,3,6] It has been suggested[11] that this antigen specific suppression regulates (and thus indicates the presence of) protective immunity in leprosy.

Hitherto studies of *M. leprae* induced immune suppression in man have been '2 stage' experiments. Suppressor cells have been generated by exposure of cell cultures to antigens of *M. leprae*: their effect has been measured by incorporating the cells into a second culture. In the present study we re-exposed healthy leprosy contacts to *M. leprae* in standard dosage by Mitsuda lepromin testing them, and assessed the resulting immune suppression by using the leucocyte migration inhibition test (LMIT) before and after lepromin testing to measure cell mediated responses to *M. leprae* antigens.

Materials and methods

Seven healthy members of the scientific and technical staff of Dhoolpet Leprosy Research Centre, Hyderabad, India, who had been working in close contact with leprosy patients for more than 3 years, were skin tested with Mitsuda lepromin (armadillo-derived, containing 4×10^7 bacilli per ml), and the late reaction (21 day) was recorded. Blood was drawn from these subjects twice to measure their LMIT responses, once before performing the skin test and again when the 21 day reaction was read.

The LMIT was performed exactly as described earlier.[8] This method is a modification of the original method described by Soborg & Bendixen.[10] Briefly, 7 ml of the anti-coagulated blood was added to 3 ml of 3% gelatin (Sigma Chemicals, USA) in saline in a culture tube. After thorough mixing it was kept at 37°C in an incubator for 45 min. The leucocyte-rich plasma was then aspirated to pellet the cells by centrifugation and subsequently for washing thrice. The cell pellet was resuspended in Minimum Essential Medium (MEM) (Bios, Bombay, India). Leucocyte concentration was adjusted to 3×10^7 cells/ml and the cell viability was checked with 0·25% Trypan Blue. The capillaries (Arthur Thomas Co., USA) were loaded with the leucocyte suspension and centrifuged at 1000 rpm for 5 min in a swing-out rotor centrifuge. Then, the capillaries were cut at the cell-medium interface and kept in polystyrene chambers which were filled with MEM containing 20% foetal calf serum (Microlab, Bombay, India) with or without antigen or mitogen and were sealed with cover-slips. Each test was run in triplicate. After 18 h of incubation at 37°C, the areas of migration were measured with planimetry.

The Migratory Index (MI) was calculated as follows:

$$MI = \frac{\text{Average area of migration with antigen}}{\text{Average area of migration without antigen}}$$

The stimulants used were the mitogen, Phytohaemagglutinin-P (PHA-P) obtained from Difco, USA which was used at 10 μg/ml. (In the dose-response study, this concentration gave optimal responses without agglutination of leucocytes. At lower concentrations (i.e. 1, 2 and 5 μg/ml) the LMI responses were weak while at higher concentrations (i.e. 20 μg and 25 μg/ml) agglutination of leucocytes was observed in the migration chambers.) *M. leprae* antigens, whole bacilli (MLW) and sonicated preparation (MLS) of the same batch (Batch No. AB 51) were kindly supplied by Dr R J W Rees, NIMR, London. They were used at $2{\cdot}5 \times 10^7$ bacilli/ml concentration (or equivalent concentration in the case of MLS) which were previously shown to be optimal for this system.[8]

Students 't' test was used for statistical analysis.

Results

The responses of each subject, before and after lepromin testing, to PHA, MLS and MLW are shown in Table 1. The responses to PHA were remarkably stable, and the means before and after lepromin testing were almost identical. The MLS responses were variable (4 showed little change, 3 suppression); they suggested

Table 1. Individual migratory indices of 7 healthy contacts before and after lepromin skin testing and their lepromin reaction

Subject number	PHA		MLS		MLW		Lepromin reaction (mm)
	Before	After	Before	After	Before	After	
1	0·70	0·79	0·88	0·94	0·67	0·82	7
2	0·79	0·80	1·08	1·05	0·63	1·01	10
3	0·72	0·68	0·84	0·75	0·81	0·83	7
4	0·71	0·58	0·66	0·84	0·60	1·02	6
5	0·56	0·76	0·57	1·09	0·79	0·95	6
6	0·86	0·79	0·66	0·98	0·74	1·02	10
7	1·22	1·19	0·91	0·95	0·90	1·09	3
Mean =	0·79	0·81	0·80	0·94	0·73*	*0·96	7
±	±	±	±	±	±	±	±
SE =	0·09	0·06	0·06	0·04	0·04	0·04	0·9

* Indicates significant ($P < 0{\cdot}01$) difference in mean values.

post-lepromin suppression, but the difference was not significant. However, the MLW responses were suppressed (i.e. the Migratory Indices were clearly elevated) in all subjects except one after lepromin testing, the mean figures showing a significant difference ($P < 0{\cdot}01$). When MLS and MLW results were pooled there was still suppression which was significant at $P < 0{\cdot}01$ level.

There was no correlation between the amount of suppression detected and the size of lepromin reaction.

Discussion

This preliminary study differs in 2 major ways from others which have demonstrated antigen specific immune suppression in man. Firstly, suppression was induced *in vivo*, suggesting that the previous studies were not simply detecting an *in vitro* artefact. Secondly, a different detection system, the LMIT, was used. This technically simple test measures lymphokine production, but can be much influenced by other effects. However, measurements of lymphokine production may be potentially more specific indicators of immune responses than are tests involving lymphoproliferation such as the LTT. It has been reported[5] that, compared with the LTT, the LMIT showed less cross-reactivity between leprosy and tuberculosis infections. In the present study the more marked MLW responses suggest that our subjects responded preferentially to surface antigens of the *M. leprae* preparations they received; but direct lymphokine assays will be needed to prove such discrimination between different antigens of *M. leprae*. The stability of the PHA responses indicates that the altered responses to *M. leprae* were specific and induced by the lepromin injection.

This study has demonstrated antigen specific immune suppression in subjects probably immunized against *M. leprae* by exposure, supporting the view of Stoner *et al.*[11] that such a phenomenon might be part of a normal, native protective response against infection. The possibility that this phenomenon could be used to indicate the presence of protective immunity deserves further exploration, particularly as it might be applied as part of the short term evaluation of potential anti-leprosy vaccines. In view of the demonstrated defect in antigen-specific lymphokine production by lepromatous leprosy patients,[2,7] tests that measure lymphokine production may be more suitable than the standard LTT for this purpose.

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