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Cellular and humoral immune responses to recombinant 65-kD antigen of *Mycobacterium leprae* in leprosy patients and healthy controls

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SUMMARY

Cellular and humoral immune responses to recombinant 65-kD antigen of *Mycobacterium leprae* (rML65) were studied in leprosy patients and healthy contacts from a leprosy-endemic population. Peripheral blood mononuclear cells from a considerable proportion of tuberculoid leprosy patients, healthy contacts and non-contacts showed proliferative response to rML65 *in vitro*. A strong positive correlation was observed between the responses to rML65 and bacille Calmette–Guérin (BCG) or leprosin A. Addition of recombinant IL-2 (rIL-2) enhanced the proportion of responders to rML65 considerably in all groups of leprosy patients, healthy contacts and non-contacts. Among lepromatous patients this enhancement was more pronounced in the bacterial index (BI)-negative group. These results indicate that the 65-kD antigen of *Myco. leprae* is a dominant T cell immunogen in our study population. Though lepromatous patients showed poor lymphoproliferative response to rML65, their IgG antibody levels to the same antigen were markedly high. Most of the BI-positive lepromatous patients with elevated anti-rML65 IgG levels did not show T cell reactivity even with the addition of rIL-2. On the other hand, tuberculoid leprosy patients, healthy contacts and non-contacts showed good T cell reactivity but low levels of IgG antibodies to rML65, thus indicating the presence of an inverse relationship between cell-mediated and humoral immune responses to a defined protein antigen of *Myco. leprae* in humans. A significant proportion of individuals among tuberculoid leprosy patients, healthy contacts and non-contacts showed neither T cell reactivity nor elevated levels of IgG antibody to rML65. However, in most of these subjects, a T cell response to rML65 was demonstrable with the addition of rIL-2. These results are discussed with reference to the immunoregulatory mechanisms occurring during *Myco. leprae* infection on the basis of differential activation of Th1 and Th2 subsets.

Keywords leprosy 65-kD antigen T helper subsets immunoregulation *Mycobacterium leprae*

INTRODUCTION

Leprosy continues to be a major public health problem in many parts of the world. There are two major issues in the immunology of leprosy which still remain unresolved. First, the antigens of *Mycobacterium leprae* that confer protective immunity in the majority of individuals in endemic areas have not been characterized completely. Second, the reasons why only a few individuals fail to develop protective immunity and become susceptible to *Myco. leprae* infection are not fully understood. Developments made during the last decade on antigenic characterization of *Myco. leprae* have greatly helped in understanding the immunoregulatory mechanisms in leprosy [1].

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Several protein antigens of *Myco. leprae* and *Myco. bovis* bacille Calmette–Guérin (BCG) have been made available in pure form through recombinant DNA technology [2]. Some of the recombinant mycobacterial antigens such as 65-kD, 70-kD and 18-kD proteins have been studied in considerable detail [3]. The 65-kD protein is a major antigenic constituent of mycobacteria [4]. The 65-kD antigens of *Myco. leprae*, *Myco. tuberculosis* and *Myco. bovis* BCG are almost identical in their amino acid sequence [5], and belong to the highly conserved hsp60 family of heat shock proteins [6,7].

Several MoAbs derived from *Myco. leprae*-immunized mice recognized the 65-kD antigen [8]. The mycobacterial 65-kD antigen has also been demonstrated to be a major T cell immunogen in mice [9,10]. Several studies have demonstrated the presence of T cells reactive to the mycobacterial 65-kD antigen in humans by deriving specific helper and cytotoxic T

cell clones from leprosy and tuberculosis patients and healthy volunteers [11–15]. However, population studies in leprosy patients and healthy endemic controls are required to evaluate the true immunological significance of the recombinant antigens [16]. There are only a very few such studies on cellular and humoral immune responses to the mycobacterial 65-kD antigen [17–21]. Further, both responses were not evaluated together in any of these investigations. Therefore, we studied the lymphoproliferative response and IgG and IgM antibody levels to the recombinant *Myco. leprae* 65-kD antigen simultaneously in leprosy patients and healthy controls from a leprosy-endemic population in southern India. This comprehensive study was aimed at evaluating the immunogenicity of the 65-kD antigen in the study population on the one hand, and understanding the immunoregulatory mechanisms in leprosy using a defined antigen on the other.

MATERIALS AND METHODS

Antigens

Mycobacterium leprae recombinant 65-kD antigen (rML65; batch ML65-5b) was generously supplied by Dr J. D. A. Van Embden (National Institute for Public Health and Environmental Protection, Bilthoven, The Netherlands) through the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). Soluble *Myco. leprae* antigen (leprosin A, batch CD91) was obtained from Dr R. J. W. Rees (WHO/IMMLEP/*Myco. leprae* Bank) and stored at −70°C. BCG (Danish strain 1331) was kindly provided by The Director, BCG Vaccine Laboratories, Madras, India. It was subjected to treatments as for the preparation of Dharmendra lepromin [22] and suspended in PBS at a concentration of 10^7 bacilli/ml.

Cell culture reagents

Metrizoate sodium, Hanks' balanced salt solution (HBSS), powdered culture medium RPMI 1640 and penicillin–streptomycin mixture were purchased from Sigma Chemical Co. (St Louis, MO). Ficoll 400 was purchased from Pharmacia (Uppsala, Sweden). Recombinant IL-2 (rIL-2) was a kind gift from Dr F. Sinigaglia (Hoffman La Roche, Basel, Switzerland).

Blood samples

Blood samples from leprosy patients, healthy family contacts (HFC) and healthy hospital staff (healthy hospital contacts, HHC) were collected from the leprosy hospital (Voluntary Health Services, Leprosy Project) located at Sakthinagar (Periyar District, Tamil Nadu, India). Leprosy patients were classified clinically and bacteriologically [23] into polar lepromatous (LL), borderline lepromatous (BL), mid-borderline (BB), borderline tuberculoid (BT) and polar tuberculoid (TT) patients. Patients with a history of reactions were excluded from the study. A total of 195 samples, collected from 131 leprosy patients (48 LL, 19 BL, 14 BB, 32 BT and 18 TT), 50 HFC and 14 HHC were studied. Both untreated patients and treated patients under multiple drug therapy (MDT) for periods ranging from 2 weeks to 228 weeks were included. LL and BL patients were grouped together, but segregated into bacterial index-positive (LBI^+) and BI-negative (LBI^-) lepromatous patients. Fifteen healthy non-contact (HNC) samples were derived from the students of the School of Biological Sciences, Madurai Kamaraj University, who had not had any habitual contact with leprosy patients even though they lived in an endemic area. The study subjects were selected randomly without any bias towards age or sex. However, children below 12 years and people above 70 years were not included. About 20 ml of venous blood were collected from each subject into heparinized vacutainers (Vacuette, Greiner, Germany). Sera separated from 4 ml of venous blood collected in siliconized vacutainers (Greiner) were decomplemented and frozen at −70°C.

Lymphoproliferative assays

Peripheral blood mononuclear cells (PBMC) separated over Ficoll–metrizoate density gradient [24] 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% normal human AB serum. Cultures with 10^5 cells in 200 μl final volume were stimulated with optimal concentrations of rML65 (10 μg/ml), leprosin A (10 μg/ml) or BCG (5×10^5 bacilli/ml). Even at two-fold higher concentrations, these antigens were not cytotoxic to PBMC. Triplicate cultures in 96-well flat-bottomed microtitre plates (Nunc, Roskilde, Denmark) were incubated for 6 days at 37°C in a humidified atmosphere of 5% CO_2, 95% air. Recombinant IL-2 was added at a concentration of 5 U/ml to cultures stimulated with rML65 48 h post-stimulation. Delayed addition of rIL-2 reduced the background proliferation to rIL-2 alone considerably. During the last 16 h of culture, 0·5 μCi of ^{3}H-thymidine (Bhabha Atomic Research Centre, 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, Turku, Finland).

The results are expressed as Δct/min (mean ct/min of antigen-stimulated cultures − mean ct/min of control cultures) or stimulation index (SI = mean ct/min of antigen-stimulated cultures/mean ct/min of control cultures). Responses were considered positive when SI > 3·0 and Δct/min > 3000 for rML65 and > 5000 for BCG or leprosin A, based on the range of response observed and published in the literature. Restoration of responsiveness to rML65 by exogenously added rIL-2 was considered positive when SI > 3·0 and Δct/min > 3000 as well as 50% higher than that for either antigen or rIL-2 alone.

Indirect ELISA for measuring antibody response to rML65

Antibodies of IgG and IgM isotypes to rML65 were measured by indirect ELISA as described by Meeker *et al.* [20] with minor modifications. ELISA plates (Corning Glassworks, Corning, NY) were coated with 4 μg/ml rML65 in 50 mM Tris buffer pH 8·0 (50 μl/well) at 37°C for 1 h and then at 4°C overnight. After washing with PBS containing 0·05% Tween 20 (PBS-T) wells were blocked with 200 μl of 1% gelatin (Sigma) in PBS for 1 h. Appropriate dilutions of serum samples in PBS-T containing 1% gelatin (1:100 for IgG and 1:20 for IgM) were added to the wells in duplicates (50 μl/well) and incubated for 1 h at 37°C. After washing, 50 μl of horseradish peroxidase (HRP)-conjugated anti-human IgG (1:2000) or IgM (1:1000) (Cappel, Turnhout, Belgium) were added. Plates were washed thoroughly and 50 μl of freshly prepared OPD substrate solution in citrate buffer pH 5·0 containing 0·012% H_2O_2 were added to the wells (50 μl/well). The reaction was stopped by the addition of 50 μl of 2 N H_2SO_4. Absorbance was measured at 490 nm using a Dynatech Minireader II (Dynatech Laboratories

Inc., Alexandria, VA). Pooled serum samples from healthy non-contacts (NSP) and lepromatous patients (LSP) were included every time the assay was performed to measure the interassay variation. The coefficient of variation observed for NSP and LSP after eight separate assays was less than 5% for both IgG and IgM responses. The antibody response was considered positive if the absorbance value was more than the mean + 2 s.d. of data from HNC.

Statistical analysis

Student's *t*-test for comparing the mean responses between the study groups, and regression analysis were performed using the EPISTAT statistical software package.

RESULTS

Lymphoproliferative responses to rML65, BCG and leprosin A

PBMC from a considerable proportion of individuals in the BT, TT, HFC, HHC and HNC groups showed proliferative response to rML65 *in vitro* (Fig. 1). The mean proliferative response was comparable among these groups. In contrast, lepromatous and BB patients showed a poor lymphoproliferative response to rML65. Simultaneously, we measured the lymphoproliferative response of the various study groups to BCG and leprosin A. In general, BCG elicited a stronger response than leprosin A, with 59% of the study subjects responding to the former compared with only 35·5% to the latter (Fig. 2). While most lepromatous and BB patients failed to respond to leprosin A, a significant proportion of them responded to BCG. Interestingly, HNC showed the highest level of responses to BCG and leprosin A (data not shown), as well as to rML65 (Fig. 1).

Analysis of the pooled data from all groups of leprosy patients, contacts and non-contacts showed significant positive correlation between T cell proliferative responses to rML65 and

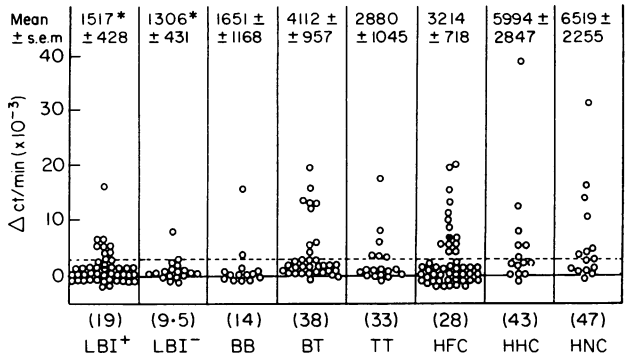

Fig. 1. Lymphoproliferative response to rML65 in leprosy patients, healthy contacts and non-contacts. Peripheral blood mononuclear cells (PBMC; 10^5) were stimulated with 10 μg/ml rML65 for 6 days. Each circle represents the response of one individual. The mean Δct/min ± s.e.m. values of the response for each group are indicated. The dashed line represents the cut-off value for positive response at Δct/min 3000. Numbers within parentheses denote the percentage of responders (Δct/min > 3000 and SI > 3·0) in each study group. ●, Non-responder with Δct/min > 3000 but SI < 3·0. Forty-eight BI-positive (LBI⁺) and 19 BI-negative (LBI⁻) lepromatous, 14 mid-borderline (BB), 32 borderline tuberculoid (BT), 18 polar tuberculoid (TT), 50 healthy family contacts (HFC), 14 healthy hospital contacts (HHC) and 15 healthy non-contacts (HNC) were studied. * Mean response significantly low compared with BT, HFC and HNC ($P < 0{\cdot}05$).

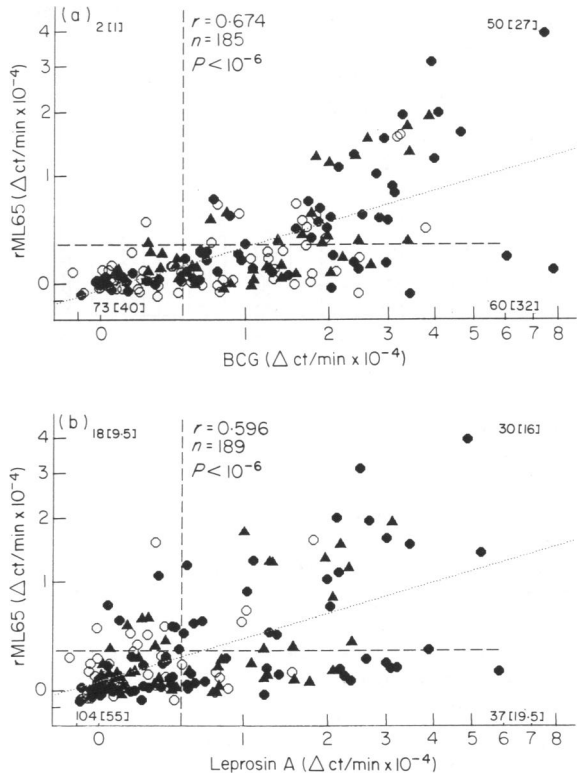

Fig. 2. Correlation between the responses to rML65 and bacille Calmette–Guérin (BCG) or leprosin A. Data from all leprosy patient groups, healthy contacts and non-contacts were pooled (a, ●, 10 healthy non-contacts (HNC), 14 healthy hospital contacts (HHC) and 44 healthy family contacts (HFC); ▲, 14 polar tuberculoid (TT) and 31 borderline tuberculoid (BT); ○, 13 mid-borderline (BB), 16 LBI⁻ and 43 LBI⁺. b, ●, 15 HNC, 8 HHC and 49 HFC; ▲, 18 TT and 29 BT; ○, 13 BB, 14 LBI⁻ and 43 LBI⁺) and correlation between the responses to rML65 and BCG or leprosin A was evaluated by regression analysis. Vertical and horizontal dashed lines bisecting the abscissa and ordinate represent the cut-off values for defining the responders to BCG or leprosin A (Δct/min > 5000 and SI > 3·0), and rML65 (see Fig. 1), respectively. 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.

BCG or leprosin A (Fig. 2). Most of the responders to rML65 were also responders to BCG and leprosin A. Quite noticeably, more than 50% of the responders to BCG (60/110) and leprosin A (37/67) did not respond to rML65.

Addition of rIL-2 to PBMC cultures stimulated with rML65 caused a marked improvement in the responsiveness to the antigen. The proportion of responders to rML65 was enhanced more than two-fold by exogeneously added rIL-2 in all groups studied (Fig. 3), and this enhancement was very pronounced among LBI⁻ patients. Comparison of the responses to rML65 and BCG or leprosin A revealed that the proportion of responders to rML65 increased upon addition of rIL-2 from 45% to 72% among BCG responders, and to 88% among leprosin A responders.

Antibody responses to rML65

Serum IgG antibody level to rML65 was significantly higher among LBI⁺ patients compared with all other patient groups,

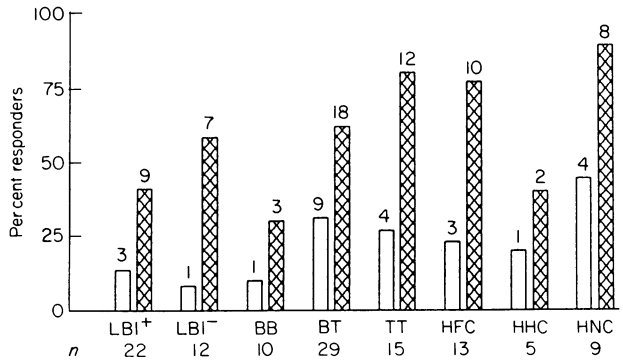

Fig. 3. Effect of the addition of rIL-2 on responsiveness to rML65 in different study groups. rIL-2 was added at a concentration of 5 U/ml 48 h after stimulation with rML65. Response to rML65+rIL-2 among rML65 non-responders was considered positive when SI > 3·0 and Δct/min > 3000 and 50% higher than the response to either rML65 or rIL-2 alone. *n*, Total number of individuals studied. Numbers above the bars indicate the total number of responders. Background response of unstimulated cultures was < 1500 ct/min and cultures stimulated with rIL-2 alone showed less than 3000 ct/min in most of the subjects. LBI, Lepromatous bacterial index; BB, mid-borderline; BT, borderline tuberculoid; TT, polar tuberculoid; HFC, healthy family contacts; HHC, healthy hospital contacts; HNC, healthy non-contacts. □, rML65 alone; ▩, rML65+rIL-2.

including LBI⁻ patients, healthy contacts and non-contacts (Table 1). Analysis of the pooled data from lepromatous patients showed significant positive correlation between anti-rML65 IgG antibody levels and BI ($r = 0{\cdot}438$, $n = 74$, $P < 0{\cdot}001$). HFC showed a significantly higher level of anti-rML65 IgG antibodies than HNC (Table 1). The anti-rML65 IgM response did not show much variation among the study groups except LBI⁻ patients.

Comparison of lymphoproliferative response and IgG antibody level to rML65

Even though the cellular immune response of lepromatous patients to rML65 was poor, about 40% of them showed high levels (above the mean +2 s.d. of HNC) of anti-rML65 IgG antibodies (Fig. 4). Even among tuberculoid patients and HFC, a small proportion of the individuals with positive IgG antibody levels failed to show lymphoproliferation. Interestingly, the majority among BT, TT, HFC, HHC and HNC groups who showed T cell reactivity were not positive for anti-rML65 IgG antibodies. Among leprosy patients and healthy contacts a significant proportion of the individuals showed neither T cell reactivity nor IgG response to rML65 (Fig. 4). However, addition of rIL-2 to PBMC cultures resulted in demonstrable T cell reactivity in most such individuals among tuberculoid patients and healthy contacts, as well as among BI-negative lepromatous patients (Table 2). It should be noted that more than 75% among the BI-positive lepromatous patients with high levels of anti-rML65 IgG antibodies failed to respond to rML65 even with rIL-2 supplementation.

DISCUSSION

For over two decades the *in vitro* lymphocyte proliferation assay has been used to measure the T cell reactivity in leprosy patients and healthy contacts. A variety of *Myco. leprae* preparations such as heat-killed or irradiated whole bacilli, Dharmendra lepromin and sonicate extract containing soluble antigens have been used [25–29]. To the above antigenic preparations, poor responses were observed even in tuberculoid patients and healthy controls, and this was attributed, at least partially, to the presence of certain immunomodulatory components such as lipoarabinomannan (LAM) [30]. This contention is supported by the T cell Western blot technique using *Myco. leprae* sonicate antigens [31], and by a recent population study using delipidified cell components (DCC) of *Myco. leprae* [32]. Further, even though there is a high degree of antigenic similarity between *Myco. leprae* and BCG, a much better T cell reactivity was observed to the sonicate antigens of the latter than to the former (Fig. 2). In this context, the best approach would be to study the immune responses to defined antigens of *Myco. leprae*. Therefore, we used the recombinant 65-kD antigen of *Myco. leprae* to study cellular and humoral immune responses of leprosy patients and endemic controls from southern India. *In vitro* lymphoproliferative responses to rML65 were observed in a considerable proportion of tuberculoid leprosy patients, healthy contacts and non-contacts. A similar finding has been reported for the 65-kD antigen of BCG [17,18]. Compared with these reports, the proportion of responders to rML65 is low in our study, and this may quite well be due to the stringent criteria we have used for defining the positive response. However, there is a general agreement on the immunogenicity of the 65-kD antigen, whether it be from BCG or *Myco. leprae*, in humans.

The present population study involving more than 200 samples from leprosy patients and endemic controls clearly demonstrates that lepromatous patients responded poorly to rML65 (Fig. 1) and to rMB65 [33]. This is also true for leprosin A (Fig. 2) and DCC [32]. However, the existence of antigen (rML65)-specific T cells in their peripheral circulation is well indicated by the pronounced enhancement in the responsiveness to rML65 upon addition of rIL-2 to the PBMC cultures of LBI⁻ as well as LBI⁺ patients (Fig. 3). Thus, in lepromatous patients, even though antigen-specific T cells exist, their reactivity is impaired even to purified mycobacterial protein antigens. This may be due to a low frequency of antigen-specific T cells in the peripheral compartment, probably resulting from their sequestration in lesions and draining lymph nodes [34–36], modulation of CD2 on T cell surface [22,37], and/or the inhibitory effect of certain *Myco. leprae* components such as LAM [30,38].

It has been known for some time that anti-*Myco. leprae* antibodies of IgG isotype are elevated in lepromatous patients while the cell-mediated immunity (CMI) against *Myco. leprae* is depressed [39]. We now demonstrate that a clear inverse relationship exists between the cellular and humoral immune responses to a defined protein antigen of *Myco. leprae* in individual subjects during natural infection. In most lepromatous patients with elevated levels of anti-rML65 IgG antibodies, the lymphoproliferative response to the same antigen was specifically depressed (Fig. 4), and was not restored even with the addition of exogenous rIL-2 (Table 2). This inverse relationship is also true for tuberculoid patients and HFC, wherein the majority who showed T cell reactivity to rML65 with or without exogenously added rIL-2 did not have elevated antibody levels to rML65 (Fig. 4, Table 2). It is thus clear that certain specific immunoregulatory mechanisms operate during *Myco. leprae* infection, resulting in the skewing of the response towards either humoral or cellular immunity.

Table 1. IgG and IgM antibody responses to rML65 in leprosy patients, healthy contacts and non-contacts

Study group	*n*	IgG response: Mean OD +s.d.	IgG response: Per cent positive responders*	IgM response: Mean OD +s.d.	IgM response: Per cent positive responders*
LBI$^+$	56	0·59+0·34†	55	0·27+0·15	7
LBI$^-$	18	0·42+0·15‡	22	0·41+0·18¶	28
BB	18	0·37+0·21	22	0·31+0·15	11
BT	30	0·35+0·19	17	0·32+0·20	13
TT	22	0·28+0·16	9	0·23+0·14	5
HFC	67	0·34+0·18§	10	0·32+0·17	9
HHC	15	0·28+0·15	7	0·33+0·33	20
HNC	47	0·28+0·13	6	0·27+0·13	2

* Responders were defined as individuals showing OD values above the mean+2 s.d. of healthy non-contacts (HNC).

† Mean value significantly higher than all other groups ($P<0·01$ to $<10^{-6}$).

‡ Mean value significantly higher than HNC, healthy hospital contacts (HHC) and polar tuberculoid (TT) patients ($P<0·05$ to $<0·005$).

§ Mean value significantly higher than HNC ($P<0·05$).

¶ Mean value significantly higher than lepromatous bacterial index-positive (LBI$^+$), borderline tuberculoid (BT), TT and HNC ($P<0·05$ to $<0·001$).

ELISA plates were coated with 4 μg/ml of rML65. Serum samples were added to the wells (1:100 for IgG and 1:20 for IgM response) followed by horseradish peroxidase (HRP)-conjugated anti-human IgG or IgM. *n*, Number of samples studied in each group.

BB, Mid-borderline; HFC, healthy family contacts.

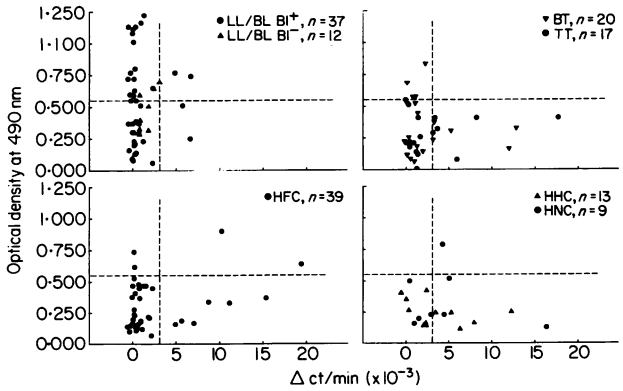

Fig. 4. Comparison of lymphoproliferative and IgG antibody responses to rML65 in leprosy patients, healthy contacts and healthy non-contacts. Vertical and horizontal lines bisecting the abscissa and ordinate represent the cut-off values for positive lymphoproliferative response and IgG antibody level to rML65, respectively (see Fig. 1 and Table 1). LL, Polar lepromatous; BL, borderline lepromatous; BT, borderline tuberculoid; TT, polar tuberculoid; HFC, healthy family contacts; HHC, healthy hospital contacts; HNC, healthy non-contacts.

Both HFC and HNC are endemic controls; however, these groups differ significantly in many respects. The proportion of individuals with T cell reactivity to rML65 is markedly low among HFC (28%) compared with HNC (47%), though the former group is constantly exposed to leprosy patients (Fig. 1). Similar differences were observed between HFC and HNC in their lymphoproliferative responses to total antigenic preparations of *Myco. leprae* and BCG [33]. The IgG antibody response to rML65 was significantly elevated among HFC compared with HNC (Table 1). Further, the proportion of IgG3 subclass antibodies to leprosin A was significantly higher in HFC than in HNC [40]. These results, together with the inverse relationship between the antibody levels and lymphoproliferative response to rML65 in HFC, indicate that the immune alterations may actually precede the development of visible lesions. In order to evaluate the validity of this contention, it would be necessary to follow up those individuals among HFC with elevated levels of anti-rML65 IgG antibodies but with poor T cell reactivity to the same antigen for the development of lesions. A similar approach had already been made in household contacts using other criteria, such as lepromin skin test reactivity and fluorescent leprosy antibody absorption test [41].

The findings of markedly high levels of anti-rML65 IgG antibodies (Table 1) and of IgG3 subclass antibodies to leprosin A [40] in lepromatous patients indicate that antigen-specific T cell help which is essential for immunoglobulin class switch [42] must be available in the system. However, T cell reactivity as assessed by the lymphoproliferation assay was found to be poor in these patients (Figs 1 and 2). The mechanism of enhanced IgG antibody production to rML65 in lepromatous leprosy in the absence of demonstrable T cell reactivity is not understood at present. It is possible that the balance between Th1 and Th2 subsets, which differentially promote cellular or humoral immune responses by lymphokines secreted by them [43], might be perturbed in lepromatous lesions. This contention is supported by the observations that (i) lepromatous T cells are defective in IL-2 and interferon-gamma (IFN-γ) secretion [40,44,45]; (ii) mycobacterial LAM inhibits IL-2 synthesis by T cells [38] as well as enhances IL-6 and IL-10 gene expression in macrophages [46]; and (iii) cytokine mRNAs of Th2 type are

Table 2. Comparison of the IgG antibody and lymphoproliferative responses to rML65 among leprosy patients and healthy contacts

		Anti-rML65 IgG-positive				Anti-rML65 IgG-negative			
			Lymphoproliferative assay				Lymphoproliferative assay		
			Per cent responders				Per cent responders		
Subjects	*n*	Per cent total	rML65 alone	rML65 +rIL-2	Per cent non-responders	Per cent total	rML65 alone	rML65 +rIL-2	Per cent non-responders
LBI$^+$	14	64	0	14	50	36	0	14	22
LBI$^-$	8	25	0	25	0	75	12·5	37	25
BB	8	25	0	12·5	12·5	75	12·5	0	62·5
BT	18	17	0	11	6	83	22	22	39
TT	14	0	0	0	0	100	21·5	57	21·5
HFC	13	15	15	0	0	85	8	54	23
HHC	5	0	0	0	0	100	20	40	40
HNC	7	14	14	0	0	86	57	29	0

Cut-off value for positive anti-rML65 IgG level is defined in Table 1. Criteria for defining the responders to either rML65 alone or rML65+rIL-2 in lymphoproliferative assays are given in Figs 1 and 3. The values given are the percentages of the total number of individuals studied in each category. *n*, Number of samples studied in each group.

LBI, Lepromatous bacterial index; BB, mid-borderline; BT, borderline tuberculoid; TT, polar tuberculoid; HFC, healthy family contacts; HHC, healthy hospital contacts; HNC, healthy non-contacts.

predominant in lepromatous lesions [47]. Therefore, it appears that there is specific inhibition of Th1 cells, with the resultant loss of their regulatory role on Th2 response and/or preferential activation of Th2 cells by *Myco. leprae* in the microenvironment of lepromatous lesions.

In conclusion, the present comprehensive study on the profile of immune responses to the 65-kD antigen of *Myco. leprae* in a leprosy-endemic population provides strong evidence for differential activation of T helper subsets at a functional level during *Myco. leprae* infection in humans.

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