

Leprosy Reactions: Humoral and Cellular Immune Responses to *M. leprae*, 65kDa, 28kDa, and 18 kDa Antigens¹

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ABSTRACT

This study examines the immune responses against some stress proteins of *Mycobacterium leprae* in leprosy patients with and without leprosy reactions. Leprosy patients showed a higher level of antibodies to all antigens compared to healthy controls. The antibody response to 18kDa antigen was significantly higher in patients with Type 1 reaction compared to those of TT or borderline patients without Type 1 reaction, or those with Type 2 reaction. Borderline (BT/BL), lepromatous (LL) and patients with reactions (Type 1 and Type 2) had higher levels of antibodies to *M. leprae* soluble extract (MLSE) and 65kDa than those of the tuberculoid (TT) group. LL, borderline patients, and patients with Type 1 reaction had a higher level of antibody to 28kDa than those of healthy controls. However, no significant differences could be observed in antibody response to these antigens (MLSE, 65kDa, and 28kDa) between patients with reaction and without reaction. A significant proportion of TT/BT patients showed positive lymphoproliferative response to MLSE compared to BL/LL patients. In addition, the lymphoproliferative response to MLSE was significantly greater in patients with Type 1 reaction compared to patients without reaction. No difference in proliferative response to 65kDa could be observed in any of these groups. The finding of high levels of antibodies against stress proteins in patients with Type 1 reactions, especially to 18 kDa antigen, along with a heightened lymphoproliferative response to MLSE is suggestive of a coexistence of cell mediated and humoral immunity in leprosy patients during Type 1 reactions. On the other hand, in Type 2 reactions no significant role of stress proteins could be demonstrated except a heightened lymphoproliferative response to the 28 kDa antigen.

RÉSUMÉ

Cette étude présente les réponses immunitaires chez les patients hanséniens et les patients souffrant de réactions, contre les protéines de stress de *Mycobacterium leprae*. Les patients hanséniens ont montré de plus hauts niveaux d'anticorps dirigés contre tous les antigènes que les personnes témoins en bonne santé. La réponse sérique dirigée contre l'antigène de 18kDa était significativement plus élevée chez les patients souffrant de réaction de type 1 comparée à celles des patients TT ou borderline, ou celle des patients avec réaction de type 2. De plus, un plus grand pourcentage de patients avec réaction inverse avaient une réponse détectable pour cet anticorps, comparé à celui des patients sans réaction. Les patients borderline (BT/BL), lépromateux (LL) et les patients avec réactions (type 1 et type 2) présentaient de plus hauts niveaux d'anticorps dirigés contre l'extrait soluble de *M. leprae* (MLSE) et la protéine 65kDa que les patients tuberculoïdes (TT). Les patients LL, borderline et les patients présentant une réaction de type 1 présentaient de plus hauts niveaux d'anticorps contre la protéine 28kDa par rapport aux témoins en bonne santé. Cependant, aucune différence significative ne fut observée entre les patients avec et sans réaction dans la réponse sérique contre les antigènes MLSE, 65kDa et 28kDa. Comparé aux patients BL/LL, une proportion significative de patients TT/BT montraient une réponse lymphoproliférative positive contre MLSE. De plus, comparé aux patients sans réaction en cours, les patients de type 1 montraient une réponse lymphoproliférative plus élevée contre MLSE. Aucune différence de réponse proliférative contre 65kDa ne fut observée entre les groupes. La mise en évidence de hauts niveaux d'anticorps dirigés contre les protéines de stress de *M. leprae* chez les pa-

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tients souffrant de réactions de type 1, en particuliers contre l'antigène 18kDa, accompagnant une réponse lymphoproliférative élevée contre MLSE, suggère qu'une immunité à médiation cellulaire et une immunité à médiation humorale coexistent chez les patients hanseniens pendant les réactions de type 1. A l'opposé, aucun rôle significatif des protéines de stress ne fut montré pour les réactions de type 2, à l'exception d'une réponse lymphoproliférative plus élevée contre l'antigène de 28kDa.

RESUMEN

En este estudio se examinó la respuesta inmunitaria contra algunas proteínas de estrés de *Mycobacterium leprae* en pacientes con lepra con y sin reacción leprosa. Comparados con los controles sanos, los pacientes con lepra mostraron altos niveles de anticuerpos contra todos los antígenos probados. La respuesta en anticuerpos contra el antígeno de 18 kDa fue significativamente mayor en los pacientes con reacción Tipo I que en los pacientes con lepra TT, los pacientes con lepra subpolar sin reacción Tipo 1, o los pacientes con reacción Tipo 2. Los pacientes subpolares (BT/BL), los lepromatosos (LL) y aquellos sin reacciones (tipos 1 y 2) tuvieron niveles más elevados de anticuerpos contra un extracto soluble de *M. leprae* (MLSE) y contra la proteína de 65 kDa, que los pacientes del grupo tuberculoide (TT). Los pacientes LL, los subpolares, y los pacientes con reacción Tipo 1 tuvieron mayores niveles de anticuerpos contra la proteína de 28 kDa que los controles sanos. Sin embargo, no se observaron diferencias significativas en la respuesta en anticuerpos contra los antígenos (MLSE, 65 Kda, y 28 kDa) entre los pacientes con reacción y aquellos sin reacción. Por otro lado, comparados con los pacientes BL/LL, una proporción significativa de los pacientes TT/BT mostraron una respuesta linfoproliferativa positiva contra el MLSE. Además, la respuesta linfoproliferativa contra el MLSE fue significativamente mayor en los pacientes con reacción Tipo 1 que en los pacientes sin reacción. En ninguno de los grupos se observó diferencia en la respuesta proliferativa contra la proteína de 65 kDa. Así, los hallazgos de niveles elevados de anticuerpos contra las proteínas de estrés en los pacientes con reacción Tipo 1, especialmente contra la proteína de 18 kDa, junto con la elevada respuesta proliferativa contra el MLSE, sugieren la coexistencia de la inmunidad celular y la inmunidad humoral en los pacientes con lepra durante la reacción Tipo 1. En las reacciones de Tipo 2 no se pudo demostrar un papel significativo de las proteínas de estrés, excepto por la marcada respuesta linfoproliferativa contra el antígeno de 28 kDa.

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. After infection the response of host immune system determines the course of the disease. On the basis of cellular immune response of the host, a spectrum of types of leprosy has been described (27). Some types of leprosy patients suffer from immunological complications known as "lepra reactions" which include both Type 1 (reversal reactions, RR) and Type 2 (erythema nodosum leprosum, ENL) reactions (14). Type 1 reaction is characterized by episodes of increased inflammatory activity in skin and in nerves of patients with borderline leprosy [Borderline tuberculoid (BT), Borderline (BB), and Borderline lepromatous (BL)] (5,31). Type 2 reaction or ENL reaction is the most serious immunological complication in BL and lepromatous (LL) patients. Type 1 reaction is known to be due to changes in (both up and down regulation) of cell mediated immunity (CMI), whereas in Type 2, there is a

rise in immune complexes with their deposition in tissues (40). Further, in Type 2 reactions a transient rise of CMI with the expression of Th 1 type of cytokines has also been noted (19,35).

Several stress proteins of *M. leprae* have been cloned (42) and are recognized by both murine and human immune cells (22,24). *M. leprae* heat shock protein (hsp) 65 kDa, expressed in macrophages transfected with the mycobacterial gene, is known to be presented to T cells in association with both MHC class I and class II (32) and also MHC non-restricted manner (33). Beimnet, *et al.* (1996) also reported the expression of Hsp 60 from a human monocytic cell line infected with *M. leprae* (2). Further, these stress proteins are known to be major antigens of mycobacteria, which induce specific antibody and T cell immune response during infections (1, 6, 8,10, 11, 14, 16, 21, 28, 29, 30, 37, 41). Nerve and skin damage in leprosy is reported to be associated with increased lev-

els of intra-lesional hsp (¹⁶). The process of reaction evokes a change in immunological status of the host leading to stress conditions for bacteria, which might result in release of stress proteins. However, the role of antibodies to stress proteins in Type 1 reactions has not been elucidated so far except for the observation of Klatser, *et al.* (¹⁷). To explore the immunological role of stress proteins of *M. leprae* in leprosy, analysis of circulating antibodies, and of the proliferative response of peripheral blood mononuclear cells (PBMC) to some of the stress proteins were performed in healthy controls, Tuberculoid (TT), Borderline and LL patients with Type 1 or Type 2 reactions, and in patients without reactions.

MATERIALS AND METHODS

Leprosy patients attending the out patient department of the Central JALMA Institute for Leprosy (Agra, India) were included in the study after obtaining their written consent according to the guidelines laid by the Indian Council of Medical Research, India. They were diagnosed clinically and bacteriologically and were divided into five groups across the disease spectrum according to the criteria of the Indian association of Leprologists (¹²). All these patients were clinically active and were on multi-drug therapy (MDT) during the study period.

Serum samples. Serum samples were obtained from 10 ml of blood drawn by antecubital venipuncture from 6 TT, 24 borderline (13 BT, 11 BL), 8 LL patients who were stable in their clinical manifestations, 21 BL/LL patients with ENL, and 29 BT/BL patients with Type 1 reaction. Nineteen laboratory volunteers who were hospital contacts served as healthy controls.

Soluble and recombinant antigens of *M. leprae*. *M. leprae* soluble extract (MLSE) (contract No-1-A1-55262) was obtained from Dr. P. J. Brennan, Colorado State University, U.S.A., and the recombinant proteins of *M. leprae* (ML hsp65kDa, ML 28 kDa, ML 18kDa) were gifted by Dr. M. Singh, GBH, Germany.

Enzyme linked immuno sorbent assay (ELISA). Maxisorp (Nunc, Roskilde, Denmark) plates were coated with 100 microlitre (μ l) antigen solutions [MLSE (2 μ g/ml), 65 kDa (1 μ g/ml), 28 kDa (2 μ g/ml)

and 18 kDa (2 μ g/ml)] in carbonate bicarbonate buffer (pH 9.2) and kept for 4 hrs at 37°C and then overnight at 4°C. The wells were blocked in phosphate buffered saline (PBS) with 3% Bovine Serum Albumin (BSA) for 1 hr at 37°C. One hundred μ l of 100-fold diluted sera in PBS containing 0.05% Tween 20 and 1% BSA was added in duplicate wells. After 2 hrs incubation at 37°C, the plates were washed 3 times in PBS Tween (0.05%). One hundred μ l of 5000-fold diluted horseradish peroxidase-conjugated anti-human IgG antibody (Sigma, St. Louis) was added, and plates were incubated for 1 hr 30 minutes at 37°C followed by a final 3 washes. One hundred μ l of orthophenylene diamine hydrochloride solution (0.5 mg/ml substrate in distilled water containing 30 μ l of 30% H₂O₂) was added to each well for development of color. The reaction was stopped after 30 minutes by adding 25 μ l of 3N H₂SO₄. The optical density (OD) was measured in an ELISA reader at 492 nm.

Peripheral blood mononuclear cells (PBMCs). Peripheral venous blood was collected aseptically in a heparinized tube from 2 TT, 22 borderline (10 BT, 12 BL), 7 LL patients who were stable in their clinical manifestations and 12 BL/LL patients with Type 2, 12 BT/BL patients with Type 1, and from 12 healthy controls. PBMCs were separated by ficoll hypaque density gradient centrifugation. The cells were collected from the interface layer and washed three times with RPMI 1640 and counted in a Neubaur's chamber.

Proliferation of peripheral blood mononuclear cells (Lymphocyte transformation test, LTT). PBMCs (2 \times 10⁶ cells/ml) were cultured in quadruplicate wells in RPMI 1640 containing 10% human AB serum in 96-well plates (Nunc, Roskilde, Denmark) for six days. Optimum concentrations of MLSE (1 μ g/ml) and different recombinant proteins [65kDa (5 μ g/ml), 28 kDa (10 μ g/ml)], were added to wells for sensitization of PBMCs. DNA synthesis was assayed by [³H] labeled thymidine incorporation (Amersham, U.K.). 1 μ Ci of ³H-thymidine (specific activity 5.0 Ci / m mol) was added to each well on 5th day and after 18 hr cells were harvested. The lymphocyte stimulation index (SI) was calculated using a standard formula (average cpm in the presence

TABLE 1. Mean level of antibodies (with standard deviations) to antigens of *M. leprae* of healthy controls and leprosy patients.

Antigens	HC N = 19	TT N = 6	Borderline (BT/BL) N = 24	LL N = 8	ENL N = 21	RR N = 29
MLSE	0.23 ± 0.13	0.30 ± 0.06	0.51 ± 0.36 [∇]	0.55 ± 0.19 [∇]	0.58 ± 0.37 [∇]	0.51 ± 0.22 [∇]
ML65kDa	0.19 ± 0.08	0.22 ± 0.04	0.29 ± 0.13 [#]	0.37 ± 0.16 [#]	0.30 ± 0.13 [#]	0.35 ± 0.22 [#]
ML28kDa	0.17 ± 0.08	0.30 ± 0.17	0.27 ± 0.13 [*]	0.42 ± 0.25 [*]	0.29 ± 0.13 [*]	0.38 ± 0.19 ^{*†}
ML18kDa	0.17 ± 0.08	0.23 ± 0.06 [*]	0.28 ± 0.15 [*]	0.42 ± 0.19 [*]	0.31 ± 0.22 [*]	0.46 ± 0.35 ^{**}

[∇] Significantly more than HC & TT (p < 0.005)

[#] Significantly more than HC & TT (p < 0.02)

^{*} Significantly more than HC (p < 0.0005)

[†] Significantly more than Borderline (BT/BL) and ENL (p < 0.03)

^{*} Significantly more than HC (p < 0.05)

^{*} Significantly more than TT, Borderline (BT/BL) and ENL (p < 0.005)

of antigen/average cpm in the absence of antigen).

Statistical analysis. Data were analyzed by Student's *t*-test by using MS Excel software program, Chi square test, and Fisher exact test.

RESULTS

Antibodies to MLSE, ML 65kDa, ML 28kDa and ML 18kDa. The mean antibody response (with standard deviations) of healthy individuals, all types of leprosy patients is presented in Table 1.

MLSE. It can be noted that all groups of leprosy patients showed high antibody levels to MLSE compared to healthy controls. Antibody level in BT, BL patients, and LL patients, and in patients during reactions (both Type 1 and Type 2) is significantly higher than those of TT group. There was no difference in antibody level between BT and BL patients and in patients during reversal reaction. Similarly, there was no dif-

ference in BL/LL patients, and those patients with Type 2 reactions.

65kDa. There was a higher level of antibody in BT/BL, LL and also in patients with reactions (ENL and RR) than in healthy controls and TT patients. No difference could be observed in antibody level between the groups of patients with reactions (Type 1 and Type 2) and without reactions.

28 kDa. It was also observed that TT, BT/BL and LL patients have higher level of antibody to this antigen in comparison to healthy controls. The highest level of antibody was detected in LL patients, followed by Type 1 patients. The difference between antibody levels in BT/BL patients as compared to those with Type 1 reactions was statistically significant, (p < 0.03).

18 kDa. The level of antibody to this antigen was observed to be at a higher level in all groups of leprosy patients compared to those of healthy controls. LL patients had

TABLE 2. The percentage of seropositivity for antibodies against various antigens of *M. leprae*.

Antigens	HC N = 19	TT N = 6	Borderline (BT/BL) N = 24	LL N = 8	ENL N = 21	RR N = 29
MLSE	0 (0)	0 (0)	10 (41.66)	5 (62.5) [*]	10 (47.61)	15 (51.72)
ML65kDa	1 (5.26)	0 (0)	8 (33.33)	4 (50)	6 (28.57)	11 (37.93)
ML28kDa	0 (0)	2 (33.33)	8 (33.33)	4 (50)	7 (33.33)	15 (51.72)
ML18kDa	0 (0)	0 (0)	7 (29.16)	5 (62.5) [*]	5 (23.80)	16 (55.17) [*]

Figures in parentheses () show percent positive.

Cut off values are mean OD +2 S.D. (MLSE = 0.49), (65kDa = 0.35), (28 kDa = 0.33), and (28 kDa = 0.33). Individuals having more OD values than cut off points are taken as positive.

^{*} Significantly more than TT (p < 0.03)

^{*} Significantly more than borderline group (p < 0.06)

TABLE 3. The positivity for lymphoproliferation in healthy controls and leprosy patients towards various antigens of *M. leprae*.

Antigens	HC N = 12	TT/BT* N = 12	BL/LL N = 19	ENL N = 12	RR N = 12
MLSE	11 (91.66)	6 (50)	1 (5.2)	1 (8.33)	8 (66.66)*
ML65kDa	2 (16.66)	1 (8.33)	1 (5.2)	2 (16.66)	1 (8.33)
ML28 kDa	2 (16.66)	0 (0)	0 (0)	3 (25)*	2 (16.66)

SI >2 is taken as positive response.

Figures in () show the percent positivity.

* Significantly more than BL/LL ($p < 0.007$)

* Significantly more than BT/BL ($p < 0.04$)

* Significantly more than BL/LL patients ($p < 0.06$) and BT/BL ($p < 0.02$)

significantly higher level of antibody than did TT patients. The mean antibody level to this antigen is highest in BT/BL patients during Type 1 reactions, which is significantly different from those of TT patients, BT/BL patients without reaction and patients with Type 2 reaction, ($p < 0.005$).

The percentage of seropositivity of antibodies to these antigens is shown in Table 2. It was noted that significant proportion of LL patients showed positivity for MLSE and 18kDa ($p < 0.03$) when compared to that of TT patients.

There was no significant difference in seropositivity of antibodies to these antigens amongst patients of the BT/BL groups with and without reaction. Nevertheless a significant proportion of BT/BL patients with Type 1 showed higher positivity to 18-kDa antigens only when compared to those of borderline patients without reactions ($p < 0.06$).

Lymphoproliferative response to MLSE and stress proteins of *M. leprae* (ML 65kDa & ML28kDa). The positivity for lymphoproliferation to these antigens (SI >2) in healthy controls and leprosy patients is presented in Table 3. The individual lymphoproliferative responses against these antigens are shown in The Figure (a, b, and c).

MLSE. MLSE was found to be the best inducer for proliferation of lymphocytes amongst all *M. leprae* proteins tested. All except one of the healthy controls showed a positive response to MLSE, whereas 1 BL patient, 1 BL patient with Type 2 reaction and none of the LL patients, were positive to this antigen. 8/12 (66.6%) of Type 1 reactions, and 6/12 TT/BT (50%) were positive to these antigens. The mean proliferative response of BT/BL patients with Type

1 was significantly greater compared to borderline patients without reaction ($p < 0.02$). However, there was no difference in mean proliferative response between BL/LL without reactions and Type 2 reaction patients. Further, the mean proliferative response was significantly greater in TT/BT patients compared to BL/LL patients ($p < 0.03$) and Type 2 reaction patients ($p < 0.04$). Significant proportions of TT/BT individuals showed lymphoproliferation when compared to BL/LL patients ($p < 0.007$).

Response to recombinant proteins. ML 65kDa. There was no significant difference in lymphoproliferative response to 65kDa protein amongst these groups (Table 3 and The Figure b). **ML 28kDa.** While 3/12 patients with Type 2 showed a positive response, none of the LL patients was responsive to this antigen (Table 3 and The Figure c).

DISCUSSION

When infectious agents enter the host, they may respond to the host environment by producing stress proteins. These stress proteins are important in eliciting immune response, which can lead to pathogenesis or protection in the host. Some recombinant antigens (stress proteins) have been reported to be immunologically important and induce B cell and T cell immune responses in leprosy (^{6, 8, 16, 21, 24, 30, 37, 38, 41}). Although these previous studies have been conducted to analyze the immune response in leprosy patients, only a few studies were carried out in leprosy patients with reactions. The objective of the present study was to analyze the level of antibodies and immunoproliferative response of PBMCs to MLSE and a few stress proteins of *M. lep-*

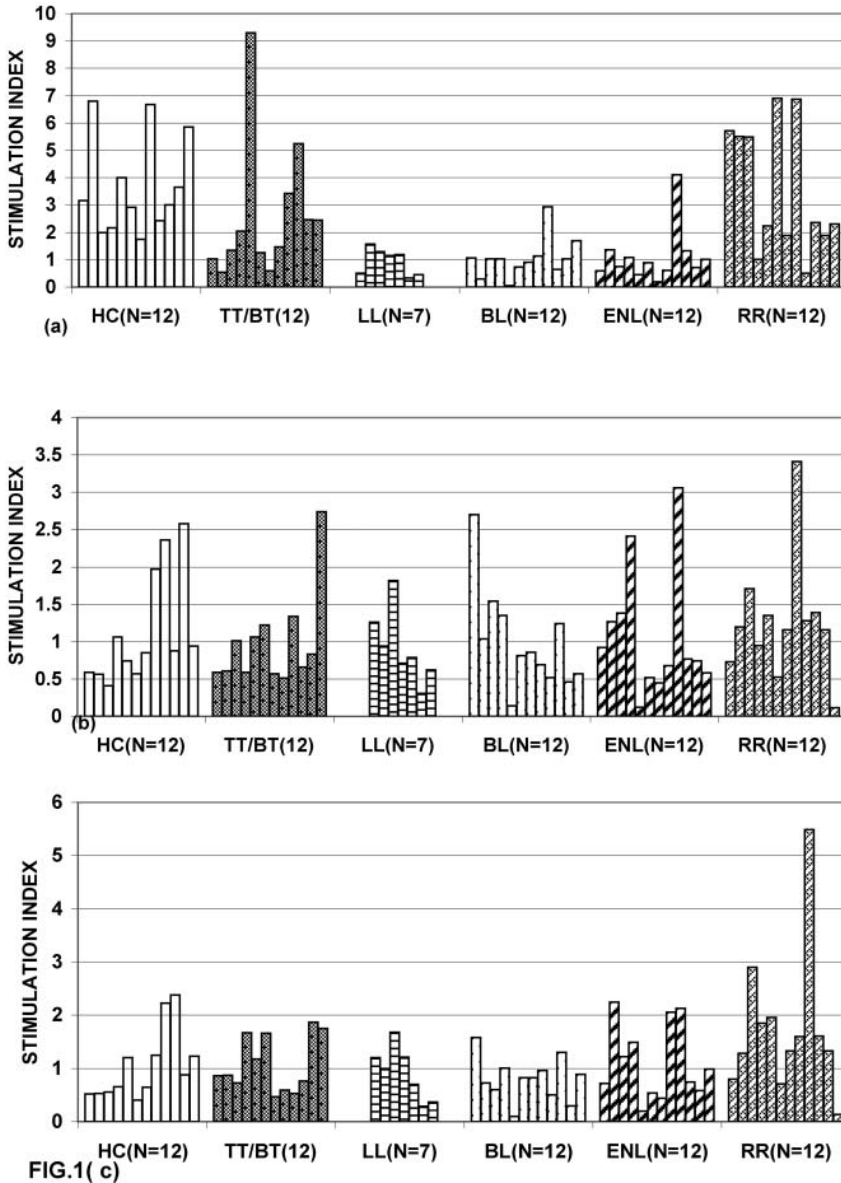


FIG.1 (c)

THE FIGURE. Proliferative response of individuals (healthy controls and leprosy patients) to MLSE (a), 65kDa (b), 28kDa(c). PBMCs (2×10^6 cells/ml) were cultured in quadruplicate wells for six days in presence of various antigens of *M. leprae* (MLSE, 65kDa, 28kDa) and in absence of antigens. Thymidine [H^3] was added after 5 days and cells were harvested after 18 hrs. Proliferative response was expressed in stimulation Index (SI). SI was calculated by using the formula (Average cpm in antigen pulsed wells/average cpm in control wells).

rae in patients associated with reactions, and to compare their levels with patients who are not associated with reactions. In addition, as controls, responses of some healthy individuals who were exposed to infection in the hospital were also compared with these leprosy patients.

Although the mean antibody level was found to be highest in patients with Type 2 reactions, this was not significantly different from other groups. Patients with Type 1 reactions also showed almost the same level of antibody to MLSE as of borderline patients. The mean level of antibodies to

MLSE was found to be significantly higher in all types of leprosy patients except TT patients when compared to those of healthy individuals. Among the patient groups, TT patients showed lowest antibody level. The mean OD value gradually increased from the TT end to the LL end of the spectrum. A similar finding has been reported earlier by Qin-xue, *et al.* (26). This finding of a gradual increase of antibody level against MLSE could possibly be due to the increase in antigenic load from the TT pole to the LL pole.

An increased level of antibodies was seen to 65 kDa, 28kDa and 18 kDa stress proteins in all groups of leprosy patients compared to healthy individuals, similar to previous reports (14, 16). In our study a higher level of anti 65kDa antibody was observed in BL/LL and Type 2 patients. However, there was no significant difference in antibody levels between patients with reaction and patients without reactions. Possibly the 65kDa antigen induces an antibody response in the initial phase of infection and this does not change during the development of various stages of disease. This observation would suggest that antibodies to 65kDa do not induce any immunopathological phenomenon in patients associated with reactions. Furthermore, the above finding is consistent with the observation of Thole, *et al.* (1995) who did not find any association with the 65kDa antigen specific responses in BT/TT or LL types of leprosy (37). Of course, *M. leprae* 65 kDa has been noted to be expressed in skin and nerve of all groups of leprosy patients (38) and may be presumed to have an important role in Type 1 reaction, but it is uncertain whether this is predominantly related to the initiation of the disease or the development of disease once the reaction has started.

We observed a higher positivity for antibody responses against the 28 kDa antigen in LL patients than TT patients, and this response was even greater in patients with Type 1 reactions. Though other studies have provided evidence of the presence of anti *M. leprae* 28 kDa antibodies in sera of lepromatous patients (6, 16), this is the first report to note such a higher percentage (51.72%) of antibody positivity to this stress protein of *M. leprae* in patients during Type 1 reactions. This antigen has

recently been suggested as a potential candidate antigen for initiating the Type 1 reaction, because it has been demonstrated in macrophages and Schwann cells of skin and nerve biopsies (20). Hence, our finding of high antibody response may be due to expression of this antigen by *M. leprae* during Type 1 reactions. Interestingly, the *M. leprae* 28 kDa protein is known to have a sequence similarity with human superoxide dismutase (SOD) (67%) and *E. coli* SOD (55%) (36). The elevated antibody level to 28kDa antigen in some of the LL and borderline patients with Type 1 reaction may be attributed to the response against increased expression of SOD in response to environmental stress during the disease process or during reaction.

The most interesting finding of our study is that the antibody level against *M. leprae* 18kDa antigen was much higher in leprosy patients with Type 1 reactions, although the percent seropositivity was also high in LL patients without reactions. Our study indicates that production of anti18-kDa antibody is a prominent event in leprosy, as all groups of leprosy patients except TT patients had a high level of antibodies to this antigen. Khan, *et al.* reported a low reactivity to this antigen in multibacillary patients (15), but we observed seropositivity of 62.5% in LL patients. Further, Roche, *et al.* (1991) observed a similar finding of a low level of anti 18kDa antibodies in paucibacillary (PB) patients and a high level in multibacillary (MB) patients (28). Many other authors have described the 18kDa protein as one of the important antigens which produces significant B cell and T cell immune response in leprosy (8, 10, 11, 24, 28). However, its association with Type 1 reactions has not been described previously. This protein was reported to have strikingly similar size and sequence to a family of heat shock proteins (25) and is expressed during heat stress (19). So, we postulate that the expression of this antigen by *M. leprae* might be increasing due to cellular resistance by the host, and as a result the host responds by producing antibodies to this stress protein. This could induce an immune response in the initial phase of infections and during Type 1 reactions.

From our observations, we conclude that circulating antibodies to some of the stress

proteins of *M. leprae* appear to play a role in Type 1 reactions. We could not observe any significant difference in antibody level against the recombinant proteins in LL patients, nor in patients with Type 2 reaction, though the reactivity was greater to some antigens in patients with Type 2 reactions than TT patients or healthy controls. Miller, *et al.* (1984) have also reported that the occurrence of Type 2 reaction had no significant effect on the total level of IgG antibody against arabinomannan (23).

With regard to the lymphoproliferative responses to these antigens, all healthy individuals except one responded to MLSE, but none of the recombinant proteins induced a strong proliferative response in this group, confirming the earlier report of Wilkinson, *et al.* (41). Moreover, most of the patients and all healthy controls were responsive to the purified protein derivative of *M. bovis* (data not included). In the present study, TT/BT patients showed stronger lymphoproliferative responses than those of BL/LL patients only to MLSE and not to recombinant proteins of *M. leprae*, consistent with the study of Thole, *et al.* (37). Further, we observed a significant lymphoproliferative response to MLSE in patients with Type 1 reaction. BJune, *et al.* (5) have already noted this with sonicated preparations of *M. leprae* in patients with Type 1 reactions. The finding of a significant lymphoproliferative response to MLSE during Type 1 reactions, compared with borderline patients without reactions, clearly indicates the upregulation of CMI in such patients.

We did not observe any significant difference in the proliferative response to 65 kDa antigen among patient groups, as reported by others. Ilangumaran, *et al.* (13) reported that there is an inverse relationship between cell mediated and humoral immune responses to 65 kDa in leprosy patients. De La Barrera, *et al.* (7) have observed that *M. leprae* 65 kDa is a poor inducer of cytotoxic T lymphocyte (CTL) in MB patients, but could induce proliferation and CTL in MB patients with Type 2 reaction.

The finding that the 28 kDa antigen induced a poor response both in TT and LL patients is in agreement with the study of Wilkinson, *et al.* (41). Lepromatous patients did not differ significantly from patients with reaction in their proliferative response to all recombinant proteins tested in our

study except that of the 28 kDa antigen where a significant proportion of patients with Type 2 reaction responded to this antigen. While a number of studies have reported the antigenic potential of 28 kDa in the humoral immune response, not much information is available regarding the nature of cell mediated response against it. Though Wilkinson, *et al.* (41) have described this as a moderate stimulator of T cell responses, they did not investigate the response in patients during leprosy reactions (Type 1 or Type 2). The significantly greater positivity in proliferative responses in Type 2 patients than those of BT/BL/LL might indicate a response to the expression of SOD during this reactional stress. The finding of a significantly greater number of Type 2 reaction cases responding to the 28 kDa antigen compared to BL/LL patients might explain their transient boost in CMI as reported earlier by other authors (18).

Although previous workers have demonstrated the presence of antibodies to these proteins in BT/TT and BL/LL patients, this appears to be the first study to demonstrate a high level of antibodies especially against 28 kDa in these patients associated with Type 1 reaction. The role of *M. leprae* antigens in Type 1 reactional pathology has been noted others (17, 20, 39). Hence, the high level of antibodies observed in patients during Type 1 reaction may be due to the *M. leprae* antigens exposed in tissues during reactions. At this moment, it is not possible to conclude whether antibodies are induced due to the development of reactional pathology, or if it has been initiated due to the induction of antibodies. The cellular immune response associated with Type 1 reaction is presumably due to other *M. leprae* antigens and not due to the stress proteins expressed by *M. leprae*.

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