*Lepr Rev* (1991) **62,** 122–133

# Association of mycobacterial-specific and *Mycobacterium leprae* specific antibody levels with clinical activity in tuberculoid leprosy: a comparative study of three serological enzyme-immunoassays

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Accepted for publication 1 December 1990

*Summary* The ELISAs for polyclonal antibodies against *Mycobacterium leprae* (ML–ELISA) and specific antibodies against epitopes on 35 kDa protein (SACT–ELISA) and phenolic glycolipid I (PG–ELISA) of *M. leprae* were evaluated comparatively in a group of 88 tuberculoid leprosy patients. The overall seropositivity rate with a battery of 3 tests (68%) was not significantly higher than that obtained with ML–ELISA alone (55%) for IgG class of antibodies. Seropositivities for SACT–ELISA and PG–ELISA were, respectively, 38% and 26%. ML–ELISA for IgM class of antibodies was least sensitive, showing only 8% positivity.

A significant correlation was noted between individual values of the three assays, but the positive proportions overlapped maximally in the case of ML–ELISA (IgG) and SACT–ELISA. Further, positivity for the latter two assays, particularly SACT–ELISA, showed significant associations with the extent of 'active' (largely untreated) infection.

Immunoblotting revealed that the main antibody response was directed towards *M. leprae* antigens in the molecular weight range of 20–40 kDa and the densitometry results of this zone correlated significantly with corresponding SACT–ELISA and ML–ELISA (IgG) values.

## Introduction

Antibody production varies in the leprosy spectrum as a consequence of graded bacillary load from tuberculoid (low bacillary load with low antibody levels) to the lepromatous (high bacillary load and high antibody levels) pole. The anti-*M. leprae* antibodies, though apparently not involved in host protection, are of significance in the serodiagnosis of leprosy and monitoring treatment.[1–4]

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The presently available *M. leprae* specific serological assays detect multibacillary (lepromatous) leprosy patients with much greater precision than the paucibacillary (tuberculoid) patients.[2] It is assumed that the tuberculoid (TT/BT) patients also produce a significant level of anti-*M. leprae* antibodies, but the available assays are not sensitive enough to detect it.[2] Nevertheless, some recent reports[5,6] indicate that 40–50% of TT/BT patients may be deficient not only in the species specific antibodies but also in antibodies to the entire range of *M. leprae* antigens.

The present study was aimed at comparing the sensitivity of two of the *M. leprae* specific antibody assays—phenolic glycolipid (PG) ELISA[7] and serum antibody competition test (for an epitope on 35 kDa *M. leprae* protein)[3,8] and an ELISA for antibodies against the soluble antigens of *M. leprae*, in tuberculoid leprosy patients. Correlations between the serology results and clinical features of individual patients were sought. Major *M. leprae* antigens recognized by sera representing a range of antibody levels (high to low) were identified by immunoblotting and densitometry.

## Materials and methods

### 1 STUDY SUBJECTS

Eighty-eight leprosy patients attending the clinics of CJIL, Agra, and classified as 'tuberculoid' (TT or BT) according to Ridley–Jopling criteria,[9] were included in the study. All patients, except two, were receiving multidrug therapy (MDT) as recommended for paucibacillary leprosy[10] for varying durations. Relevant clinical data on patients were obtained from their case records.

Eighteen healthy volunteers were included in the study as controls.

Sera were collected and stored at $-20°C$ until used.

### 2 ANTIGENS AND ANTIBODIES

Soluble antigen of *M. leprae* (stock solution = 1 mg protein/ml), prepared by sonication and high-speed centrifugation of armadillo derived *M. leprae*, was supplied by IMMLEP bank (courtesy of Dr R J W Rees). Natural disaccharide (serologically active determinant of PG-I antigen of *M. leprae*) conjugated with BSA (ND-O-BSA) was provided by Dr Delphi Chatterjee (Colorado State University, CO, USA). The monoclonal antibody MLO4, reactive with an epitope on 35 kDa protein antigen of *M. leprae*,[11–13] was provided by Dr J Ivanyi (in the form of ascites globulin) which was later conjugated to peroxidase (Sigma, Type VI) using the standard procedure.[14] Peroxidase conjugated antibodies to human immunoglobulins (whole, IgG or IgM) were obtained from Dakopatts, Denmark.

### 3 SEROLOGICAL ASSAYS

#### (a) *ELISA for antibodies to soluble antigens of* M. leprae *(ML–ELISA)*

IgG and IgM classes of antibodies were measured in individual sera. Briefly, ELISA plates (Immunoplate I, Nunc, Denmark) were coated with either *M. leprae* soluble antigens (1·25 $\mu g$ protein/50 $\mu l$/well, 4°C, overnight) diluted in coating buffer (carbonate–bicarbonate, 0·05 M, pH 9·6) or with coating buffer alone and blocked with 1% skimmed

milk powder (Anikspray, Lipton India Ltd) in tris-buffered saline (pH 7·4) containing 0·05% Tween-20 (TBS-T). Antigen coated as well as buffer coated wells were incubated in duplicate with different serum dilutions (specified below) made in 1% milk–TBS-T for 1 hr at 37°C. Washed (with TBS-T) plates were incubated with peroxidase conjugated anti-human IgG or IgM antibodies diluted 1:1000 in 1% milk–TBS-T (50 $\mu$l/well, 37°C, 1 hr). Colour was developed with ortho-phenylene diamine (OPD) substrate solution (50 $\mu$l/well, 37°C, 20 min) and reaction was stopped with 7% $H_2SO_4$ (50 $\mu$l/well). Optical densities (ODs) were read at 492 nm using an ELISA reader (Titretek multiskan plus, Flow Labs). Results are expressed as $\Delta$OD (=mean OD with antigen-coated wells–mean OD with buffer-coated wells).

A serum was considered as 'positive' when its $\Delta$OD exceeded the mean $\Delta$OD+2SD (cut-off value) of normal control sera. For deciding the working serum dilutions which would provide best discrimination along with lowest cut-off values, 3 dilutions (1:500, 1:1000 and 1:5000) of all sera were tested. The most suitable dilution for IgG class of antibodies was found to be 1:5000, giving a cut-off value of 0·11 (corresponding value at 1:500 dilution was 0·85). For IgM class of antibody assay, the dilution of 1:500 was found more suitable (cut-off OD=0·42). Subsequently, results with only these dilutions were taken into consideration.

### (b) *ELISA for antibodies to 35 kDa protein antigen*

Species specific antibodies to an epitope on 35 kDa *M. leprae* protein were assayed by serum antibody competition test (SACT–ELISA), described previously.[3,8,12] Briefly, the ELISA plates coated with soluble antigen of *M. leprae* were incubated with peroxidase conjugated monoclonal antibody-MLO4, in the presence or absence of serial ten-fold dilutions of a serum. OPD was used as substrate and percent MLO4 binding at each serum dilution calculated (100%=mean $OD_{492}$ for MLO4 binding in the absence of serum). Results are expressed as $ID_{50}$ titres (serum dilution causing 50% inhibition of MLO4 binding). On the basis of data obtained from non-leprosy control subjects,[3] and this study, an $ID_{50}$ titre of $\geq 10$ was regarded as positive.

### (c) *ELISA for antibodies to phenolic glycolipid-I (PG)*

Anti-PG antibodies were assayed by indirect ELISA according to the described procedure.[3,7] Briefly, sera (diluted 1:300) were incubated in the wells of ELISA plate coated with synthetic disaccharide-BSA conjugate (ND-O-BSA) or BSA alone. Binding of IgM type of anti-PG (ND) antibodies were detected by peroxidase conjugated anti-human IgM, using OPD as substrate. On the basis of results obtained from non-leprosy controls,[3] and this study, a serum showing $OD_{492}$ value of $> 0{\cdot}20$ was regarded positive for antibody.

## 4 SDS–PAGE, IMMUNOBLOTTING AND DENSITOMETRY

Soluble antigens of *M. leprae* (1 mg protein/ml) and molecular weight markers (BDH, Poole, UK) were subjected to SDS–PAGE (under reducing conditions) on mini slabs of 12·5% gel, according to Laemmli[15] using Bio Rad (USA) mini Protean II apparatus. After electrophoresis, the gel slabs were subjected to electroblotting[16] in Bio Rad trans-blot

apparatus (60 V × 4h, in cold store) using S & S nitrocellulose paper (NCP, pore size = 0·45 μm).

The NCP strip containing transferred molecular weight markers was stained with Amido black and the remaining NCP (containing *M. leprae* antigens) was blocked with 3% skimmed milk powder (Anikspray, Lipton India Ltd) prepared in tris-buffered saline (0·01 M tris, 0·15 M NaCl, pH 7·4) containing 0·05% Tween-20 (TBS-T) for 2 hr at room temperature (RT) with gentle rocking (5–7 rpm, on 'Rockomat II', Technomara, Switzerland). Afterwards, the paper was cut into 5 mm wide strips and incubated (2 hr at RT with gentle rocking) with different sera (diluted 1 : 10 in 1% milk TBS-T). The washed (5 times with TBS-T) strips were incubated with peroxidase conjugated anti-human immunoglobulin antibody (1 : 500, 2 hr at RT, with gentle rocking). The strips were washed again (5 times with TBS-T, finally with TBS) before treating with substrate solution (0·05% 4-chloronaphthol, 0·02% $H_2O_2$, in TBS). Colour was developed by gentle rocking and reaction was stopped by extensive washing of strips with distilled water.

Densitometry on NCP strips after immunoblotting was performed on a Shimadzu CS 930 (Japan) densitometer at 530 nm optical wavelength.

### 5 STATISTICAL ANALYSIS

Significance of correlation between the results of the 3 serological assays and densitometry readings were calculated by a computer using BMDP (USA) statistical software.

Significance of associations between seropositivity rates and clinical parameters were derived from 2 × 2 contingency tables by applying $\chi^2$ test. Probability ($p$) values $< 0{\cdot}05$ were regarded as significant.

## Results

### 1 SEROPOSITIVITY BY THREE DIFFERENT ASSAYS AND CORRELATIONS BETWEEN ANTIBODY LEVELS

Seropositivity results with ML–ELISA (IgG), SACT–ELISA, PG–ELISA and their combination are compiled in Table 1. The patients were arbitrarily divided into two major groups according to the extent of skin involvement, i.e. those with > 10 or < 10 lesions or 'patches'. Each group was further divided into three subgroups according to treatment duration: treated for (i) < 50% or (ii) > 50% of the total duration of the disease and (iii) an uncertain period. Nine patients, relevant information on whom was either incomplete or ambiguous were put in a separate 'not known' group. Forty-eight (55%), 33 (38%) and 23 (26%) patients showed positivity, respectively, for ML–ELISA, SACT and PG–ELISA. A combination of all 3 tests detected seropositivity in 60 (68%) patients, 30 of whom were positive for only one of the tests (20 for ML–ELISA and 5 each for SACT and PG–ELISAs). Only 14 (16%) patients were positive for all the 3 tests.

The difference in the rate of positivity with ML–ELISA and a combination of all the 3 tests was not significant ($d = 13$, $SE = 7{\cdot}269$, $p > 0{\cdot}05$), but that between ML–ELISA and SACT–ELISA ($d = 17$, $SE = 7{\cdot}408$, $p < 0{\cdot}05$) or PG–ELISA ($d = 29$, $SE = 7{\cdot}069$, $p < 0{\cdot}01$) was significant.

The greatest overlap in seropositivity was seen between ML–ELISA and SACT (26

**Table 1.** Seropositivity in different groups and subgroups of tuberculoid leprosy patients with the three antibody assays

| Groups | Sub-groups | $n$ | ML–ELISA (IgG) $n$ (%) | SACT–ELISA $n$ (%) | PG–ELISA $n$ (%) | Overall positivity[a] |
|---|---|---|---|---|---|---|
| > 10P[c] | < 50 T[b] | 28 | 20 (71) | 17 (61) | 10 (36) | 24 (86) |
| | > 50 T | 14 | 7 (50) | 3 (21) | 3 (21) | 9 (64) |
| | U[d] | 3 | 3 (100) | 3 (100) | 2 (67) | 3 (100) |
| < 10P | < 50 T | 22 | 10 (45) | 3 (14) | 3 (14) | 13 (59) |
| | > 50 T | 10 | 3 (30) | 3 (30) | 2 (20) | 5 (50) |
| | U | 2 | 1 (50) | 0 (0) | 0 (0) | 1 (50) |
| Not known | | 9 | 4 (44) | 4 (44) | 3 (33) | 5 (56) |
| Total | | 88 | 48 (55) | 33 (38) | 23 (26) | 60 (68) |

[a] Positive for one or more of the 3 assays.
[b] Treated for lesser or greater than 50% of the disease duration.
[c] No. of skin lesions (patches).
[d] Treatment status is uncertain.

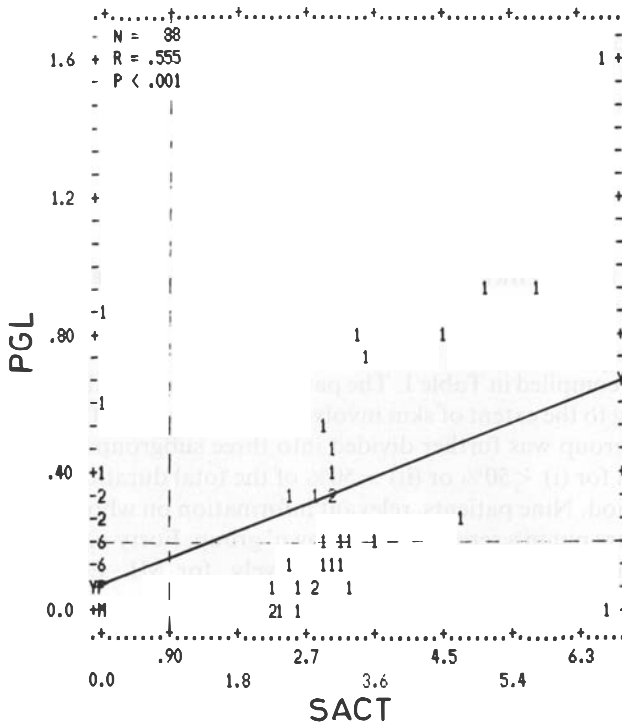


**Figure 1.** Correlation between anti-*M. leprae* antibody titres measured by PG–ELISA and SACT–ELISA in sera of 88 tuberculoid patients. PGL (PG–ELISA) values are expressed as $OD_{492}$ and SACT values are expressed as log $ID_{50}$. Dotted lines denote seropositivity cut-off values. Scattered numbers indicate one or more identical results (A = 10, B = 11, C = 12, . . .). Solid line (Y–Y) is regression line.

patients). Further, ML–ELISA levels were higher in those patients who were also positive for SACT (median = 0·52, mean = 0·67, SD = 0·55) than those who were SACT negative (median = 0·21, mean = 0·31, SD = 0·32). However, these differences were not significant statistically.

The assay for IgM class of anti-*M. leprae* antibodies showed a poor sensitivity. Only 7 (8%) patients were positive for ML–ELISA (IgM), 5 of whom were having multiple (> 10) skin lesions and were also positive for the remaining assays (ML–ELISA (IgG), SACT and PG–ELISA).

Individual antibody levels with each of the 3 assays and their mutual correlations (PG–ELISA *vs* SACT, ML–ELISA (IgG) *vs* SACT and PG–ELISA *vs* ML–ELISA (IgM), respectively) are shown in Figures 1 to 3. Correlations for remaining combinations were as follows: between PG–ELISA and ML–ELISA (IgG) $R = 0{\cdot}417$, $P < 0{\cdot}001$; ML–ELISA (IgG) and ML–ELISA (IgM): $R = 0{\cdot}134$, $p = 0{\cdot}212$ (not significant); and between SACT and ML–ELISA (IgM): $R = 0{\cdot}393$, $p < 0{\cdot}001$.

## 2 Relationship between seropositivity and extent of the disease

Two criteria were considered as indicators of the extent of infection in individual patients: the number of skin patches and the duration of treatment. The data are presented in Table 2.

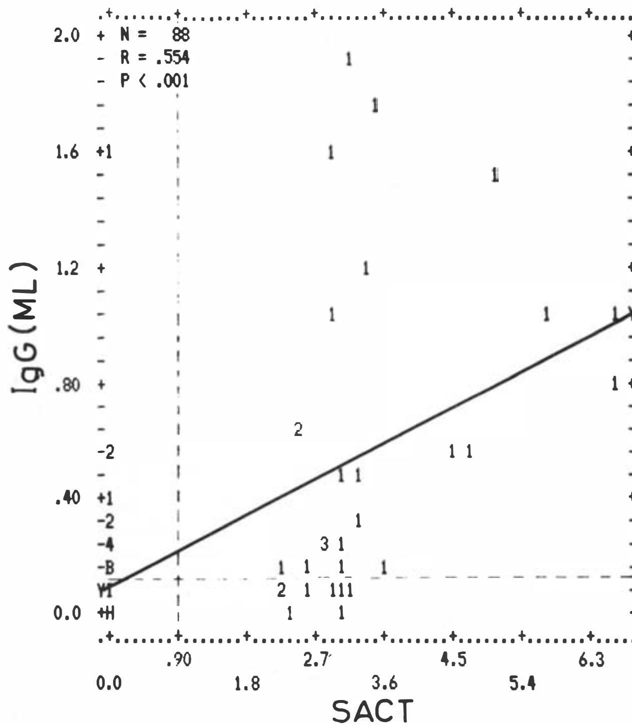


**Figure 2.** Correlation between antibody titres measured by ML–ELISA (IgG) and SACT–ELISA. IgG antibody levels are expressed as $OD_{492}$. Remaining information is given with Figure 1.

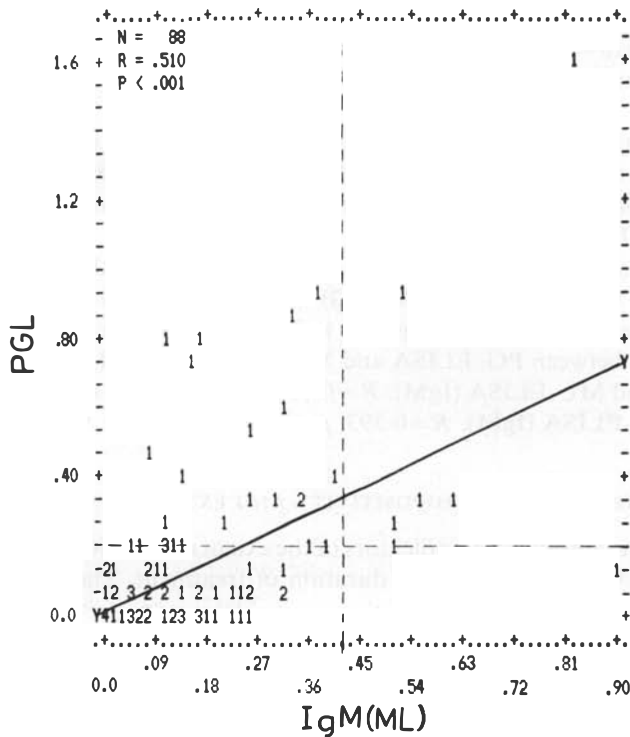


**Figure 3.** Correlation between antibody titres by PG–ELISA and ML–ELISA (IgM). Both antibody levels are expressed as $OD_{492}$. Remaining information is given with Figure 1.

**Table 2.** Association of antibody activities with the extent of the disease and strength of these associations*

| Groups | *n* | Overall | | | ML–ELISA | | | SACT–ELISA | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | + | – | %(+)[1] | + | – | %(+)[2] | + | – | %(+)[3] |
| More extensive disease (> 10P) | 45 | 36 | 9 | 80 | 30 | 15 | 67 | 23 | 22 | 51 |
| Less extensive disease (< 10P) | 34 | 19 | 15 | 56 | 14 | 20 | 41 | 6 | 28 | 18 |
| $\chi^2$ | | 5·33 ($p<0·05$) | | | 5·09 ($p<0·05$) | | | 9·33 ($p<0·01$) | | |

*No significant associations ($p>0·05$) were observed between: (i) Number of patches (P) and PG–ELISA and (ii) treatment duration (alone) and any of the 3 assays or their combination.

[1,2,3]These columns depict strength of association between positivity for antibodies and more extensive disease (> 10P) which were, respectively, 1·43, 1·63 and 2·83 times greater than the corresponding positivity in patients with < 10P.

Using a combination of all the three antibody assays, the seropositivity rate in patients having > 10 patches (80%) was significantly higher than that in patients with < 10 patches (56%). However, when associations of the number of lesions with individual test results were considered, only two of these, ML–ELISA and SACT–ELISA, were found to be

**Table 3.** Influence of treatment on overall antibody activity in tuberculoid patients with more extensive disease (> 10 patches)

| | Combination of 3 tests | | |
|---|---|---|---|
| | + | − | $\chi^2$ |
| < 50T | 24 | 4 | 6·46 ($p < 0·02$) d.f. = 1 |
| > 50T | 9 | 5 | |

statistically significant ($p < 0·05$ and $p < 0·01$, respectively). In an attempt to compare the strength of this association (Table 2), it was noted that the proportion of SACT positives in patients with more extensive disease ( > 10 patches) was 2·8 times higher than that in patients with less extensive disease ( < 10 patches); whereas the corresponding difference in the case of ML–ELISA was only 1·6 times.

Grouping of patients according to treatment status alone (treated for < 50% or > 50% of the disease duration) did not yield any significant association. Nonetheless, influence of treatment on seropositivity rates was evident from data on patients with a more extensive disease (Table 3). Of 42 such patients, combined seropositivity was significantly higher in those who were treated for < 50% of the disease duration (85·7%

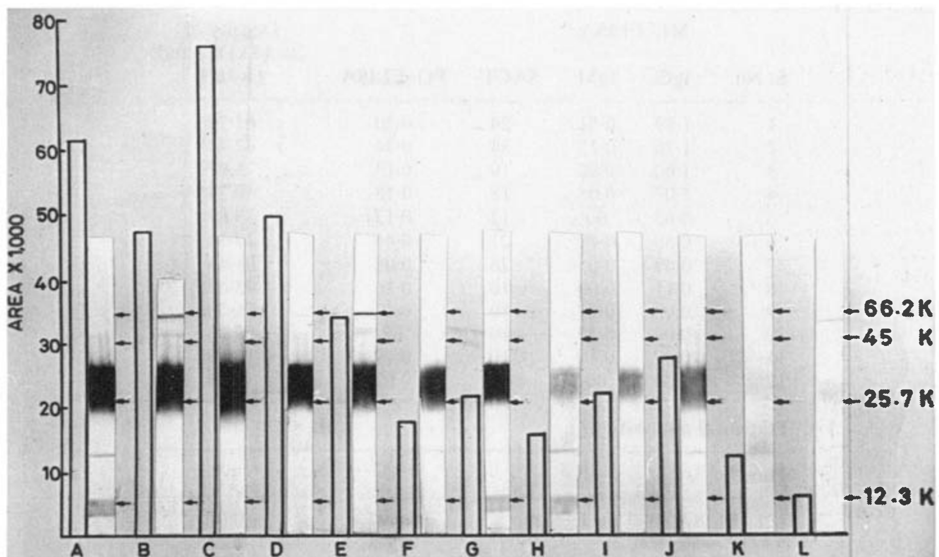


**Figure 4.** Results of SDS–PAGE followed by immunoblotting and densitometry. *M. leprae* antigens electroblotted on nitrocellulose paper (NCP) were reacted with 12 (A to L) tuberculoid leprosy sera representing a range of ML–ELISA (IgG) levels. Open bars on the left of each antibody stained NCP strip denote the density (area × 1000) of central 20–40 kDa band. Arrows on the right of each NCP strip indicate the position of molecular mass markers, whose values are given on the extreme right.

positive) than those treated for > 50% of disease duration (64·3% positive). Treatment status showed no such effect on seropositivity in patients with fewer (< 10) skin lesions.

### 3 PROFILE OF ANTI-*M. LEPRAE* ANTIBODIES AND A DENSITOMETRIC CORRELATE OF ANTIBODY LEVELS IN THE SERA

Twelve patients, representing a range of ML–ELISA (IgG) levels (high to low), were selected for the study of serum antibody profile by SDS–PAGE, immunoblotting (using *M. leprae* soluble antigen) and densitometry. Results on individual patients are compiled in Figure 4 and Table 4.

Upon immunoblotting, the major reaction appeared as a broad band in 20–40 kDa region, although with variable intensity, with all the 12 tested sera (Figure 4). The density of this band was measured in each case (represented by open bars on the left of corresponding immunoblot strips in Figure 4). As shown in Table 4, a statistically significant correlation was noted between density and antibody levels measured by ML–ELISA ($R = 0{\cdot}897$, $p < 0{\cdot}001$) and SACT–ELISA ($R = 0{\cdot}586$, $p < 0{\cdot}05$).

Other *M. leprae* antigens which reacted with this set of representative sera (Figure 4) were ≃65 kDa (with 5 sera—B, D, E, G and L) ≃18 kDa (with 3 sera—A, H and J) and ≃12 kDa (with 3 sera—A, G and H).

**Table 4.** Antibody assay and densitometry results in a selection of tuberculoid leprosy patients showing a range (high to low) of ML–ELISA (IgG) values

| S. No. | ML–ELISA IgG | ML–ELISA IgM | SACT | PG–ELISA | Density of 20–40 kDa band ($\times 10^3$) |
|---|---|---|---|---|---|
| 1 | 1·89 | 0·51 | 24 | 0·20 | 61·70 |
| 2 | 1·76 | 0·15 | 34 | 0·74 | 47·14 |
| 3 | 1·60 | 0·02 | 19 | 0·13 | 75·97 |
| 4 | 1·07 | 0·05 | 18 | 0·18 | 49·24 |
| 5 | 0·65 | 00 | 12 | 0·13 | 33·89 |
| 6 | 0·50 | 0·08 | 21 | 0·44 | 17·34 |
| 7 | 0·49 | 0·05 | 26 | 0·08 | 21·43 |
| 8 | 0·11 | 0·09 | < 10 | 0·16 | 15·62 |
| 9 | 0·08 | 0·11 | < 10 | 0·12 | 21·70 |
| 10 | 0·07 | 0·26 | < 10 | 00 | 27·23 |
| 11 | 0·07 | 0·19 | < 10 | 0·09 | 11·69 |
| 12 | 0·04 | 00 | < 10 | 0·02 | 6·16 |

Statistical analysis

| Pairs* | 'r' | 'p' |
|---|---|---|
| ML–ELISA (IgG) *vs* Density | 0·897 | < 0·001 |
| SACT *vs* Density | 0·586 | 0·044 |
| SACT *vs* ML–ELISA (IgG) | 0·813 | < 0·001 |
| PG–ELISA *vs* SACT | 0·687 | 0·012 |

*Correlations between rest of the combinations were not significant ($p > 0{\cdot}05$).

**Discussion**

The tuberculoid (TT–BT) leprosy patients harbour *M. leprae* in small numbers (due to a strong cell-mediated immunity) and hence are called 'paucibacillary'. This, and the earlier studies[1,2,6–8] show that only 30–40% of these patients are positive for *M. leprae* specific antibodies that too have low titres. Even ELISAs for polyclonal antibodies against *M. leprae* have shown positivity in the range of 50–60% (this study and[5,6]). These observations suggest that the generation of antibody response *in vivo* requires a 'threshold' level of antigen(s) which may be absent in some of the tuberculoid leprosy patients. Moreover, in the majority of such cases, the existing pool of viable infection (responsible for the generation of relevant antigens) may further be reduced by the phenomenon of immune-mediated 'self-healing'.[17]

A direct relationship between the levels of anti-*M. leprae* antibodies and the extent of viable infection is evident from significant associations of antibody positivities with greater skin involvement and lesser treatment duration. It appears that the individual levels of *M. leprae* specific antibodies such as those detected by SACT, rather than 'total' antibody levels, could be responsible for this association due to the following reasons: (a) SACT–ELISA level constitutes a part of the corresponding ML–ELISA level, since the 35 kDa antigen (responsible for positivity by SACT) is a part of the *M. leprae* soluble antigen which was used for ML–ELISA, (b) a significant correlation was observed between results of SACT and ML–ELISA, and (c) the association between more extensive disease and seropositivity rate was stronger in the case of SACT than that in the case of ML–ELISA. An association between *M. leprae* specific antibody levels and the extent of active infection has been noted on earlier occasions also, in studies involving mainly multibacillary (BL–LL) patients under treatment.[3,4]

Another considerable point of this study is an apparent parallelism in antibody levels detected by the three assays, as evident from statistically significant mutual correlations. This was seen despite the fact that two of the three assays (SACT and PG–ELISA) were based on *M. leprae* specific determinants on different antigenic molecules and the third (ML–ELISA) was having a 'broad' specificity.

An attempt to characterize the immunodominant *M. leprae* antigens responsible for seropositivity by various assays was made using immunoblotting and densitometry. The most prominent reaction was seen with *M. leprae* antigen in the region of 20–40 kDa molecular weight and the density of this band could be correlated significantly with corresponding results of SACT and ML–ELISA. Detailed analysis of the constituents in this reaction would throw light on immunodominant antigen(s) of *M. leprae*. One of the important constituents of this band, besides the 35 kDa antigen (responsible for SACT)[8,11,12] could be the cell wall associated lipoarabinomannan (30–35 kD) which is known to be one of the most potent inducers of humoral immunity in all types of leprosy.[18] Another relevant antigen in this context could be 36 kDa antigen.[19] A lack of reactivity of antibodies present in these sera with other *M. leprae* antigens (barring occasional reactions with bands at 65 kDa, 18 kDa and 12 kDa positions) suggests the futility of any future attempt to develop better serological assays based on *M. leprae* antigens hitherto untried.

In conclusion, a substantial proportion of tuberculoid patients would most probably show negativity for any type of antibody towards *M. leprae* for reasons discussed above. Thus, *M. leprae* antibody based serology is not likely to be useful in the diagnosis of

paucibacillary leprosy. Nevertheless, the observed dependence of certain antibody levels on the quantum of 'active' infection in the body can judiciously be exploited for monitoring these patients.

## Acknowledgment

We are grateful to Dr R J W Rees for providing *M. leprae* antigen, Dr J Ivanyi for monoclonal antibodies to *M. leprae* and Dr Delphi Chatterjee for ND-O-BSA. We also thank Mr P N Sharma and Mr R Dayal for technical assistance, Mr H O Agarwal for photography and Mr Anil Kumar Chopra for secretarial assistance. The manuscript was kindly reviewed by Dr H Srinivasan. Some materials used in this study were generously given by Lepra, UK.

## References


1. Gaylord H, Brennan PJ. Leprosy and the leprosy bacillus: recent development in characterization of antigens and immunology of the disease. *Ann Rev Microbiol*, 1987; **41:** 645–75.
2. Anonymous. Serological tests for leprosy. *Lancet*, 1986; **(i):** 533–5.
3. Sinha S, McEntegart A, Girdhar BK, Bhatia AS, Sengupta U. Appraisal of two *Mycobacterium leprae* specific serological assays for monitoring chemotherapy in lepromatous (LL/BL) leprosy patients. *Int J Lepr*, 1989; **57:** 24–32.
4. Klaster PR, de Wit MYL, Fajardo TT, Cellona RV, Abalos RN, de la Curz EC, Madarang MG, Hirsch DS, Douglas JT. Evaluation of *M. leprae* antigen in the monitoring of a dapsone based chemotherapy of previously untreated lepromatous patients in Cebu, Philippines. *Lepr Rev*, 1989; **60:** 178–86.
5. Touw JML, Diepen TW, Harboe M, Belehu A. Relation between anti-*Mycobacterium leprae* antibody activity and clinical features in borderline tuberculoid (BT) leprosy. *Int J Lepr*, 1983; **51(3):** 305–11.
6. Bach M-A, Wallach D, Flageul B, Hoffen-bach A, Cottenot I. Antibodies to phenolic glycolipid I and to whole *M. leprae* in leprosy patients: evolution during therapy. *Int J Lepr*, 1986; **54:** 256–67.
7. Cho SN, Fujiwara T, Hunter SW, Rea TH, Gelber RH, Brennan PJ. Use of an artificial antigen containing the 3,6-di-O-methyl-$\beta$-D-glucopyranosyl epitope for the serodiagnosis of leprosy. *J Infec Dis*, 1984; **150:** 311–22.
8. Sinha S, Sengupta U, Ramu G, Ivanyi J. Serological survey of leprosy and control subjects by a monoclonal antibody-based immunoassay. *Int J Lepr*, 1985; **53:** 33–8.
9. Ridley DS, Jopling WH. Classification of leprosy according to immunity: a five group system. *Int J Lepr*, 1986; **34:** 255–73.
10. WHO Study Group. *Chemotherapy of leprosy for control programmes.* Technical Report Series No. 675. WHO: Geneva, 1982.
11. Ivanyi J, Sinha S, Aston R, Cussell D, Keen M, Sengupta U. Definition of species specific and cross reactive antigenic determinants of *Mycobacterium leprae* using monoclonal antibodies. *Clin exp Imm*, 1983; **52:** 528–36.
12. Ivanyi J, Morris JA, Keen M. Studies with monoclonal antibodies to myco-bacteria. In: *Monoclonal Antibodies Against Bacteria.* Macario, AJL, Macario, EC (eds), New York: Academic Press, 1985; 59–90.
13. Hunter SW, Brennan PJ. The native protein of *M. leprae*: preliminary results on subcellular location, isolation and chemical and immunological characterization. Paper presented in 24th US–Japan Joint Leprosy Research Conference, San Diego, 23–25 August, 1989, Abs in *Int J Lepr*, 1990; **58:** 190–1.
14. Weir, DM. Enzyme immunoassays: heterogeneous and homogeneous systems. *Handbook of Experimental Immunology*, Vol. I, Edn 4th. USA: Blackwell Scientific Publications, 1986, 27.16.
15. Laemmli UK. Clevage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature*, 1970; **277:** 680–5.
16. Towbin H, Staehalin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. *Proc Natl Acad Sci USA*, 1979; **76:** 4350–4.
17. Lara CB, Nolasco JC. Self healing or abortive and residual forms of childhood leprosy and their probable significance. *Int J Lepr*, 1956; **24:** 245–63.
18. Brennan PJ. Lipid and carbohydrate antigens of *M. leprae*. *Lepr Rev*, 1986; **57** (Suppl. 2): 39–51.
19. Klaster PR, de Wit MYL, Kolk AHJ. An ELISA-inhibition test for the serology of leprosy. *Clin exp Imm*, 1985; **62:** 468–73.

## Association des niveaux des anticorps spécifiques aux mycobactéries et au *Mycobacterium leprae* avec l'activité clinique dans la lèpre tuberculoïde: une étude rélative de trois immunoessais sérologiques enzimatiques

VINITA CHATURVEDI, SUDHIR SINHA, B K GIRDHAR, K KATOCH, A S BHATIA ET U SENGUPTA

*Sommaire* Une évaluation rélative des essais EISA pour des anticorps policlonales contre le *Mycobacterium leprae* (ELISA-ML) et des anticorps spécifiques contre des sites antigéniques sur la protéine de 35 kDa (ELISA-SACT) et le glycolipide phénolique I (ELISA-PG) de *M. leprae* a étémenéchezun groupe de 88 patients de lèpre tuberculoïde. Le taux global de séropositivité (d'un 68%) calculé à partir d'une série de trois essais n'a pas été notamment plus élevé que celui calculé à partir d'un ELISA-SACT seul pour le classe des anticorps IgG. Les séropositivités obtenues par l'ELISA-SACT et l'ELISA-PG étaient d'un 38% et d'un 26% respectivement. L'ELISA-ML pour la classe des anticorps IgM fut le moins sensible, avec un taux de positivité d'un 8% seulement.

Les valeurs individuels obtenus à partir des trois essais ont montré une corrélation importante, mais les proportions des résultats positifs empiétaient au maximum dans le cas de l'ELISA-ML (IgG) et l'ELISA-SACT. En plus, le taux de séropositivité pour les deux derniers essais, en particulier pour l'ELISA-SACT, ont montré des associations significatives avec l'étendue de l'infection 'active' (c'est à dire dans une large mesure non-traitée).

Des transférences immunologiques ('immunoblotting') ont révélé que la réaction principale des anticorps était dirigée contre des antigènes de *M. leprae* avec des poids moléculaires compris entre les 20 et les 40 kDa et les résultats de la densitométrie dans cette zone ont montré une corrélation importante avec les valeurs corréspondants des ELISA-SACT et ELISA-ML (IgG).

## Associación de niveles de anticuerpos específicos a las micobacterias y de anticuerpos específicos a la *Mycobacterium leprae* con la actividad clínica en la lepra tuberculoide: estudio relativo de immunoensayos serológicos enzimáticos

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*Resumen* Se llevó a cabo una evaluación relativa de ensayos EISA para anticuerpos policlonales contra la *Mycobacterium leprae* (ELISA-ML) y de anticuerpos específicos contra sitios antigénicos en la proteína de 35 kDa (ELISA-SACT) y en el glicolípido fenólico I (ELISA-PG) de *M. leprae* en un grupo de 88 pacientes de lepra tuberculoide. El índice global de reacción seropositiva (de un 68%) obtenido con una serie de tres ensayos no resultó ser mucho más elevado que aquel obtenido utilizando el ELISA-ML a solas con anticuerpos de la clase IgG (de un 55%). Los índices de reacción seropositiva obtenidos con el ELISA-SACT y con el ELISA-PG fueron de un 38% y de un 26% respectivamente. El ELISA-ML para anticuerpos de la clase IgM resultó ser el menos sensible, con un índice de reacción seropositiva de sólo un 8%.

Se observó una correlación importante entre los valores individuales obtenidos en los tres ensayos, pero las proporciones de resultados positivos coincidieron al máximo en el caso del ELISA-ML (IgG) y del ELISA-SACT. Es más, en estos últimos dos ensayos, en particular en el ELISA-SACT, la reacción positiva mostró asociaciones importantes con la amplitude de la infección 'activa' (es decir, en su mayor parte no tratada).

Transferencias immunológicas ('immunoblotting') llevadas a cabo mostraron que la reacción principal de los anticuerpos fué contra antígenos con pesos moleculares comprendidos entre los 20 y los 40 kDa y los resultados de densitometría en esta zona mostraron una correlación importante con los valores obtenidos por medio de los ensayos ELISA-SACT y ELISA-ML (IgG).