that take into consideration the immunological dimensions of the disease and tread a therapeutic path that ensures the least encounter with immunological phenomena that downgrades the disease or prolongs the treatment.

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Evaluation of MLPA Test for the Serodiagnosis of Leprosy

TO THE EDITOR:

An enzyme-linked immunosorbent assay (ELISA) using *Mycobacterium leprae* cell wall phenolic glycolipid-I (PGL-I) ([1, 3, 12]) and a serum antibody competition test (SACT) based on the 35-kDa([10]) and 36-kDa([6]) protein antigens of *M. leprae* may be some of the most reliable serological tests available today for the detection of *M. leprae* infection. ELISA has been used widely in serological studies probably due to the easy availability of PGL-I antigen through the World Health Organization (WHO) in a semisynthetic disaccharide octyl bovine serum albumin (ND-O-BSA) form. However, ELISA has little field application since it can be performed only in a laboratory with expensive equipment such as an ELISA reader. Attempts to simplify this technique for field use as the dot ELISA ([13]) or stick ELISA([7]) have been of little success. Nevertheless, a serological test called the *M. leprae* particle agglutination (MLPA) test that is applicable to the field has recently been described by Izumi and his colleagues([5]). In order to assess the usefulness of the MLPA test, we compared this test with the PGL-I ELISA and SACT using monoclonal antibody to the 35-kDa protein antigen.

Serum samples for this study were collected from 188 leprosy patients, 34 healthy occupational contacts, and 48 healthy noncontacts. The patients were classified according to Ridley and Jopling ([8]), and the numbers of serum samples in each patient group are given in Table 1. At the time of blood collection they were undergoing multidrug therapy (MDT) for periods ranging from 6 months to 1 year but had skin lesions suggestive of active disease. The MLPA test was performed using the Serodia-Leprae kit (Fujirebio Inc., Tokyo, Japan). Serum samples were diluted to 1:16 and 1:32 in 96-well, U-bottom microtiter plates. Twenty-five μl of unsensitized gelatin particles and 25 μl gelatin particles sensitized with synthetic trisaccharide of PGL-I (NT-P-BSA) were mixed with 25 μl of 1:16- and 25 μl 1:32-diluted serum samples, respectively. After being incubated for 2 hr at room temperature, the plates were read for agglutination. Serum samples showing agglutination at the 1:32 dilution of sera were considered positive. ELISA with ND-O-BSA antigen, the synthetic disaccharide of PGL-I (kindly supplied by IMMLEP, WHO) was carried out as described in our previous paper ([4]). Serum samples were tested at the 1:300 dilution, and those showing OD values $\geq$ 0.200 were considered positive. The SACT was done following the method of Sinha, *et al.* ([10]) but using peroxidase- instead of isotope-labeled monoclonals. Serum samples were applied at the 1:10 dilution and those causing 50% inhibition of MLO4 binding to *M. leprae* sonicate (ID50) were considered positive.

Table 1 summarizes the percent positivity for each test in different groups of subjects. Overall seropositivity rates were found to be 51%, 54%, and 42% for the MLPA test, ELISA, and SACT, respectively. These rates increased to 65%, 70%, and 57% when sera of patients were considered separately. Also, when we analyzed the patients showing positivity to any one of the tests, the seropositivity increased to 82%, suggesting that simultaneous application of all three tests could detect most of the *M. leprae* infections. In general, the seropositivity rates of the three tests did not show much difference in multibacillary patients. However, in

TABLE 1. *Seropositivity rates to tests in different groups of sera.*

Group	No. sera tested	MLPA positive	ELISA positive	SACT positive	Any of three tests
		No. (%)	No. (%)	No. (%)	No. (%)
Tuberculoid	19	7 (37)	7 (37)	4 (21)	9 (47)
Borderline tuberculoid	48	22 (46)	23 (48)	10 (21)	29 (60)
Borderline borderline	8	5 (63)	5 (63)	4 (50)	7 (88)
Borderline lepromatous	24	21 (88)	22 (92)	20 (83)	23 (83)
Lepromatous	89	68 (76)	74 (83)	70 (79)	87 (98)
Occupational contacts	34	8 (24)	8 (24)	2 (6)	13 (38)
Noncontact	48	8 (17)	6 (13)	3 (6)	14 (29)
Total in all	270	139 (51)	145 (54)	113 (42)[a]	182 (67)
Total in patients	188	123 (65)	131 (70)	108 (57)	155 (82)

[a] Significantly ($p < 0.05$) lower than MLPA and ELISA.

paucibacillary patients even though the seropositivity rates were more or less similar for the MLPA test (43%) and the ELISA (45%), it was much lower (21%) for the SACT, indicating that the prevalence of antibodies to the 35-kDa antigen of *M. leprae* in paucibacillary patients is limited. It was also found that all three tests could give concordant results only in 64% of the serum samples (Table 2). Slightly higher concordances were noticed when two of the tests were compared. While the MLPA test and ELISA showed 76% concordance, the MLPA test and SACT and the ELISA and SACT showed 65% and 67%, respectively. The results in 20% of sera, however, showed no concordance between the tests. While the MLPA test (65.4%) and ELISA (69.7%) showed more or less similar sensitivity, the sensitivity of the SACT (57%) was observed to be lower than the other two although it was not statistically significant.

Similar to this study, others have also observed lower ELISA ([1, 3, 12]) and SACT ([10]) positivity in paucibacillary leprosy patients as compared to multibacillary patients. However, in contrast to our results, Roche *et al.* ([9]) have reported higher positivity to the SACT (33%) and lower positivity to ELISA (20%) in a group of paucibacillary leprosy patients from Nepal. This is surprising because, unlike ours, the patients included in their study were untreated and, as such, are supposed to show more positivity. It appears that this discrepancy is not due to the cut-off value employed to determine ELISA positivity since both studies have used 0.200 OD as the cut-off mark. A study involving both of these tests in untreated leprosy patients from a different area may throw some light on this point.

Our results suggest that the MLPA test is equally efficient in detecting *M. leprae* infection. Although the concordance between

TABLE 2. *Concordance between and among the three tests.*

Group	No. sera tested	MLPA ELISA SACT	MLPA ELISA	MLPA SACT	ELISA SACT
		No. (%)	No. (%)	No. (%)	No. (%)
Tuberculoid	19	13 (68)	16 (84)	13 (68)	13 (68)
Borderline tuberculoid	48	26 (54)	36 (75)	27 (56)	27 (56)
Borderline borderline	8	4 (50)	5 (62)	4 (50)	4 (50)
Borderline lepromatous	24	19 (79)	22 (92)	19 (79)	20 (83)
Lepromatous	89	55 (62)	63 (71)	58 (65)	63 (71)
Occupational contacts	34	21 (62)	26 (76)	21 (62)	21 (62)
Noncontact	48	34 (71)	37 (77)	34 (71)	34 (71)
Total in all	270	172 (64)	205 (76)	176 (65)	182 (67)
Total in patients	188	117 (62)	142 (76)	121 (64)	127 (68)

the MLPA test and the ELISA in this study (76%) is relatively less than that reported (89%–100%) by Izumi, *et al.* ([5]), it may partly be due to the different antigens used in the ELISA. Whereas synthetic disaccharide (ND-O-BSA) has been employed in our ELISA, Izumi, *et al.* ([5]) have used synthetic trisaccharide (NT-P-BSA) similar to the one used for the MLPA test. These disaccharide and trisaccharide antigens show some difference in binding with anti-PGL-I antibodies, as already reported ([2]). Recently, Wu, *et al.* ([11]) have also reported the development of an agglutination test by sensitizing latex particles with native PGL-I and ND-O-BSA. Although attempts were made to sensitize latex particles with ND-O-BSA in our laboratory, the results were not encouraging because the sensitization did not last for long.

Since the performance of the MLPA test is easier than the ELISA, it is highly suitable for screening populations for *M. leprae* infection, permitting more samples to be handled per day. A further advantage is that the results of this test can be assessed with the naked eye and, therefore, it can be performed in the field or in peripheral laboratories. We believe that the introduction of this test in place of the ELISA in ongoing seroepidemiological studies would enable the investigator to screen more of the population with a sensitivity corresponding to the ELISA. However, the question of whether the serological studies would really be useful in detecting leprosy in the population, because the infection often heals naturally, can be answered only if the results of the longitudinal epidemiological studies currently underway in several parts of the world are reported. Nonetheless, since it has been shown that the MLPA test gives titer values similar to the ELISA it may have greater application in monitoring the effect of multidrug therapy in leprosy patients.

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Does Previous BCG Vaccination Interfere with the Serodiagnosis of Tuberculosis Using *Mycobacterium tuberculosis*-specific Glycolipid Antigens?

TO THE EDITOR:

There is evidence to the effect that the development of protective T-cell-mediated protective immunity results in suppression of humoral B-cell immunity in leprosy and tuberculosis ([4]). BCG vaccination is believed to induce protective immunity against tuberculosis. It may be reasonably assumed that BCG-vaccinated individuals who subsequently are diagnosed with tuberculosis should build up protective immunity more efficiently than nonBCG-vaccinated individuals. Therefore the sensitivity of serodiagnosis should be lower in BCG-vaccinated tuberculosis patients. To examine this question, we decided to conduct a study in patients residing where BCG coverage was wide and where the incidence of tuberculosis was high as a means of detecting sufficient numbers of tuberculous patients who had received BCG vaccination.

The city of Manaus (Amazonas, Brazil) was selected for the study because, according to the health authorities, about 50% of the population had been vaccinated 1 month after birth and the incidence of smear-positive cases of tuberculosis was estimated at 69.2/100,000 inhabitants for the last decade (ranging from 70.5 in 1981 to 58.9 in 1989). During the same period, the population in the state of Amazonas increased by about 30% (1.5 million in 1981 and 2.0 million in 1989).

As part of the clinical history of all patients who are personally observed at the National Research Institute of the Amazonia (INPA), previous BCG vaccination is registered. Evidence of BCG vaccination is established by searching for the characteristic vaccinal scar in the deltoid region. This scar is easily differentiated from the smallpox vaccination scar which can still be found in the older age groups. The frequency distribution of 273 confirmed tuberculosis cases in respect to previous BCG vaccination and decade of life is shown in The Figure. The proportion of patients who received BCG vaccination was 48.3% and, as shown in The Figure, the proportion of vaccinated patients was over 50% during the first four decades of life, dropping sharply thereafter. Judging from these unexpected findings, we decided that a sample of about 50 successive cases of tuberculosis should be enough to answer the question of whether previous BCG vaccination might interfere with the sensitivity of serodiagnosis. As shown in The Figure, the sampled population was representative of the whole population of tuberculosis patients.

Tuberculosis disease was confirmed in all cases indicated above by isolation and identification of *Mycobacterium tuberculosis* using current methods ([3]). To start the study, blood was collected from successive patients before treatment, and the sera were kept frozen until ready to use. When the total number of bacteriologically confirmed tuberculosis cases reached 53 their sera were used for ELISA. The serology procedure used was as described previously ([1]). The antigens used were the PGL-Tb1 and the SL-IV glycolipids isolated from the strain Cannetti of *M. tuberculosis* ([2, 7, 8]). ELISA