

# ANTI-PGL-1 ANTIBODY LEVELS IN THAI LEPROSY PATIENTS

Kowit Kampirapap and Namthip Singtham

Sasakawa Research Building, Raj-Pracha-Samasai Institute, Nonthaburi 11000, Thailand

**Abstract.** Phenolic glycolipid - 1 (PGL-1) is a *Mycobacterium leprae* specific cell wall component. It is an immunodominant antigen and can induce a strong humoral immune response. IgM antibody levels against PGL-1 were measured in Thai leprosy patients between October 1992-April 1994 by a commercially available *M. leprae* particle agglutination test (MLPA).

The percentage of seropositivity was much higher in newly untreated multibacillary (MB) patients (83.9%) than in paucibacillary (PB) patients (17.8%). Antibody levels in the MB group varied in the range 32-8,192, whereas they varied in the range 32-256 in the PB group. Patients being treated with multidrug therapy (MDT) were 68.3% and 19.4% seropositive in the MB and PB groups, respectively. Seropositivities in control serum specimens were 11.3% in active pulmonary tuberculosis patients, 2.6% in dermatologic patients and 4.4% in a healthy population.

In conclusion, the anti-PGL-1 assay using MLPA appears to be a sensitive and specific diagnostic tool for the diagnosis of MB patients. Additionally, it may provide an alternative to the BI determination in monitoring MB patients under MDT, and also in the surveillance of such patients after MDT.

## INTRODUCTION

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. The organisms primarily affect skin and cutaneous nerves causing deformities and many social problems. The disease has a long incubation period, and it requires a long term regular multidrug treatment (MDT) of at least 6 months to 2 years or even more (Becx-Bleumink, 1991; Katoch *et al*, 1989; Dasananjali, 1989) as well as long term surveillance of at least 3 years to ensure that the disease has been cured. In spite of effective multidrug therapy against leprosy and careful follow up, relapse have been observed (Becx-Bleumink, 1992; Boerrigter *et al*, 1991; Grugni *et al*, 1990; Kurg *et al*, 1989; Li, 1993; Reddy and Mohinuddin 1988; Van Brakel *et al*, 1989).

There is, at the moment, no available effective vaccine against leprosy. The main strategy for controlling leprosy at the present and to achieve the goal of the elimination of leprosy as a public health problem by the year 2000 still relies on early detection and effective treatment of cases with multidrug therapy (Noordeen, 1991). The development of a simple and rapid diagnostic test for leprosy is therefore justified, particularly for the identification of those who are infected but have not yet developed any symptoms of disease, or those who have clinically nondiagnostic signs. Early treatment of this

group would be able to prevent deformities and reduce the risk of spreading the disease in the community by eliminating them as a source of transmission. This technique must be simple, sensitive and specific enough to be applicable in field work.

Several studies have been carried out to identify the specific antigenic determinants on the cell wall of *M. leprae* (Brennan, 1986). One of these, phenolic glycolipid-1 (PGL-1) (Hunter *et al*, 1982) has been extensively studied (Brennan and Barrow, 1980) and chemically synthesized (Fujiwara *et al*, 1984). PGL-1 is a highly specific antigen of *Mycobacterium leprae*.

The trisaccharide segment of the PGL-1 has been synthesized in the form of trisaccharide-phenyl propionate coupled to bovine serum albumin (NT-P-BSA) (Fujiwara and Izumi, 1987). This synthetic antigen has then been tested for its reactivity and specificity for leprosy sera by enzyme-linked immunosorbent assay (ELISA) and by *M. leprae* particle agglutination using gelatin particles (MLPA) (Izumi *et al*, 1988; Sugiyama *et al*, 1988; Chanteau *et al*, 1989). It seems promising to use this antigen and both detection methods for the serodiagnosis of leprosy. Although ELISA is a specific and sensitive method, it is quite expensive and rather sophisticated. However, MLPA, using Serodia-Leprae (Fujirebio Inc, Tokyo, Japan) is so

simple, reliable and inexpensive that it can be carried out in the field and in countries where technical facilities are too limited for setting up the ELISA method.

The objective of this study was therefore to conduct a seroepidemiological survey of anti-PGL-1 antibody titers in new and old cases of leprosy patients to assess the test for possible early diagnosis of subclinically infected or clinically undetermined cases by MLPA.

## MATERIALS AND METHODS

### Study populations

Four institutions contributed to this study. Raj-Pracha-Samasai Institute, Bangkhaen Skin Clinic, Bangkok Skin Clinic and Phra-Pradaeng Hospital contributed 55, 90, 58 and 55 leprosy patients, respectively (total 258 cases) between October 1992 until April 1994. The classification (Ridley and Jopling, 1966) of submitted cases was 119 lepromatous (LL), 53 borderline lepromatous (BL), 4 mid borderline (BB), 51 borderline tuberculoid (BT), 26 tuberculoid (TT) and 5 indeterminate (I) (Table 1). All leprosy patients were about to begin treatment or were already being treated with the WHO recommended regimen of multidrug therapy. In this regimen paucibacillary (PB) cases [I, TT, BT (BI negative)] received supervised rifampicin 600 mg once monthly, and DDS 100 mg daily for at least 6 months. Multibacillary (MB) cases [BT (BI posi-

tive), BB, BL, LL] received supervised rifampicin 600 mg once monthly, supervised clofazimine 300 mg once monthly, clofazimine 50 mg once daily, and DDS 100 mg once daily for at least 2 years.

Controls were composed of 468 active pulmonary tuberculosis patients from the Tuberculosis Division, Department of Communicable Disease Control, Ministry of Public Health, 77 patients presented with skin diseases other than leprosy at Bangkhaen Skin Clinic and 179 healthy population from the Department of Preventive Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University.

### Detection of antibodies to PGL-1

A volume of 5 ml whole blood was drawn from each subject, from which 2 ml of serum was separated and transferred to the Sasakawa Research Building, Ministry of Public Health, for measurement of level of antibodies to PGL-1 by MLPA.

**Qualitative assay:** Serum dilutions of 1:8 and 1:16 were prepared by depositing 3 drops (75 µl) of serum diluent in well 1 and 1 drop (25 µl) each in well 2 and 3 of a 96-well U-type microtiter plate using a calibrated pipette dropper. 25 µl of each serum specimen was placed in well 1 and thoroughly mixed; 25 µl was transferred from well 1 to well 2, and subsequently from well 2 to well 3. The excess 25 µl from well 3 was discarded. One drop of unsensitized particles was added to well 2 and 1 drop of sensitized particles was added to well 3 using the drop-

Table 1  
Classification of leprosy patients.

Institutes	No. of MB patients					No. of PB patients				Grand total
	LL	BL	BB	BT	Total	BT	TT	I	Total	
A	44	4	2	-	50	2	2	1	5	55
B	36	16	-	5	57	26	6	1	33	90
C	15	16	1	1	33	11	11	3	25	58
D	24	17	1	-	42	6	7	-	13	55
Total	119	53	4	6	182	45	26	5	76	258

A = Raj-Pracha-Samasai Institute  
B = Bangkhaen Skin Clinic

C = Bangkok Skin Clinic  
D = Phra-Pradaeng Hospital

pers supplied in the kit. A microplate mixer was used to mix the fluid of the wells thoroughly. Plates were covered and allowed to stand at room temperature for 2 hours. Upon completion of the reaction the settling patterns were read with the naked eye. Specimens which were reactive with sensitized particles (showing significantly large ring with a rough outer margin and agglutination in the periphery, or with a filmy mat of homogeneous agglutination covering the entire bottom of well) and which were also nonreactive with unsensitized particles (showing compact button or compact ring with a smooth round outer margin) were interpreted as positive in the qualitative test.

**Quantitative test:** A semiquantitative test was performed by further serial two-fold dilution to find the end point of the positive reaction. The antibody titer was expressed as the highest dilution giving complete agglutination. In this study, 1:32 was established as a cut-off value, defining a serum specimen which showed agglutination with less than 1:32 final serum dilution negative and that with equal to or more than 1:32 positive.

## RESULTS

Among newly diagnosed, untreated leprosy patients (Table 2), 47 out of 56 MB (83.9%) and 8 out of 45 PB (17.8%) were seropositive. High antibody titers up to 1:8,192 were found in 32 LL (94.1%).

Titers up to 1:4,096 were found in 14 BL (73.7%), 1:512 in 1 BT-MB (100%), up to 1:256 in 5 BT-PB (20.8%), 1:32 in 2 I (50.0%) and 1 TT patient (5.9%). In the same study, both BB (100%) were seronegative.

Among leprosy patients who were receiving MDT (Table 3), 86 out of 126 MB (68.3%) and 6 out of 31 PB (19.4%) were seropositive. Antibody titers up to 1:16,384 were found in 62 LL (72.9%), up to 1:1,024 in 20 BL (58.8%), up to 1:512 in 2 BB (100%), 1:32 in 2 BT-MB (40.0%), and up to 1:512 in 6 BT-PB patients (28.6%). All 9 TT patients and the single I patient were seronegative.

Table 4 shows the results in controls. Of 468 active pulmonary tuberculosis patients, 53 (11.3%) were tested positive by MLPA. Two (2.6%) dermatologic patients and 8 (4.4%) healthy persons were also seropositive in low titers (1:32-1:64).

## DISCUSSION

Antibodies to PGL-1 antigen reflect a specific marker of *Mycobacterium leprae* infection. There have been several studies supporting this hypothesis (Buchanan *et al*, 1983; Dissanayake *et al*, 1984; Cho *et al*, 1983; Gonzalez-Abreau and Gonzalez, 1987). This study was the first undertaken in the Bangkok metropolitan area. IgM antibody to the semisynthetic trisaccharide moiety of

Table 2  
Seroreactivity to PGL-1 in sera from new leprosy patients before MDT.

Type	No. assayed	Seropositive to PGL-1		Antibody titer									
		No.	%	32	64	128	256	512	1024	2048	4096	8192-	
MB - LL	34	32	94.1	1	3	3	1	4	10	6	2	2	
	- BL	19	14	73.7	2	2	1	6	0	2	0	1	0
	- BB	2	0	0	0	0	0	0	0	0	0	0	0
	- BT	1	1	100.0	0	0	0	0	1	0	0	0	0
Total	56	47	83.9	3	5	4	7	5	12	6	3	2	
PB - BT	24	5	20.8	1	1	2	1	0	0	0	0	0	0
	- TT	17	1	5.9	1	0	0	0	0	0	0	0	0
	- I	4	2	50.0	2	0	0	0	0	0	0	0	0
Total	45	8	17.8	4	1	2	1	0	0	0	0	0	0

Table 3

Seroreactivity to PGL-1 in sera from patients under chemotherapy.

Type	No. assayed	Seropositive to PGL-1		Antibody titer								
		No.	%	32	64	128	256	512	1024	2048	4096	8192-
MB - LL	85	62	72.9	11	14	13	2	7	8	3	2	2
- BL	34	20	58.8	5	2	5	3	2	3	0	0	0
- BB	2	2	100.0	0	1	0	0	1	0	0	0	0
- BT	5	2	40.0	2	0	0	0	0	0	0	0	0
Total	126	86	68.3	18	17	18	5	10	11	3	2	2
PB - BT	21	6	28.6	3	1	0	1	1	0	0	0	0
- TT	9	0	0	0	0	0	0	0	0	0	0	0
- I	1	0	0	0	0	0	0	0	0	0	0	0
Total	31	6	19.4	3	1	0	1	1	0	0	0	0

PGL-1 was examined in both newly diagnosed, untreated patients and in leprosy patients already undergoing treatment. Percentages of seropositivity were much higher in new MB leprosy patients (83.9%) than in PB patients (17.8%). Antibody titers in the MB group varied from 1:32 to 1:8,192, whereas they varied from 1:32 to 1:256 in the PB group. This trend was also observed in patients under chemotherapy but to a lesser degree. Thus, a direct correlation has been found between the antibody titer and the severity and activity of leprosy. MLPA seems to be more liable to detect anti-PGL-1 antibodies in the multibacillary cases of leprosy. Specificity of MLPA has been also supported by the results in control samples; 11.3% TB patients, 2.6% dermatologic patients and 4.4% healthy persons were tested positive, mostly with low titers (1:32-1:64). This demonstrates that IgM antibody to PGL-1 detected by MLPA is a useful marker for sero-epidemiological surveys and can assist in the diagnosis and possible classification of leprosy, particularly multibacillary form. Cases showing high antibody titers without cardinal signs of leprosy should be kept under close observation because of a possibility of subclinical infection. The possibility of employing MLPA for monitoring the success of multidrug therapy in multi-bacillary leprosy patients should be further investigated. Additionally, it might be an alternative to the BI determination for the surveillance of multi-bacillary patients after multidrug therapy.

Table 4

Seroreactivity to PGL-1 in the active pulmonary tuberculosis patients, dermatologic patients and healthy persons.

Type	No. assayed	Seropositive to PGL-1	
		No.	%
TB	468	53	11.3
Dermatology	77	2	2.6
Healthy	179	8	4.4

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#### ANTI-PGL-1 IN LEPROSY

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