

## Detection of Phenolic Glycolipid I of *Mycobacterium leprae* and Antibodies to the Antigen in Sera from Leprosy Patients and Their Contacts

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Serum specimens from leprosy patients, their contacts, and controls were examined for the presence of phenolic glycolipid I (PGL-I), a *Mycobacterium leprae* specific antigen, and antibodies to the antigen using enzyme-linked immunosorbent assays. Of 12 lepromatous patients with less than 2 years of therapy, 11 (91.7%) were seropositive to PGL-I, thus indicating that new lepromatous cases can be identified by detecting anti-PGL-I antibodies. In contrast, 88 (56.4%) of 156 lepromatous patients treated more than 2 years were positive. Moreover, only 69 (40.8%) were seropositive among 169 lepromatous patients in the leprosy resettlement villages. The mean antibody level also declined significantly in proportion to the duration of chemotherapy. This may suggest the possibility of monitoring chemotherapy by detecting anti-PGL-I antibodies. The prevalence of anti-PGL-I antibodies among 200 controls from a high endemic area for leprosy was 5.5% and was significantly higher than that (1.5%) among 200 controls from a low endemic area. Of 103 household contacts in the resettlement villages, 10 (9.7%) were seropositive, reflecting the frequent chance of exposure to *M. leprae*. However, PGL-I was not detected in any of the sera from controls, contacts, and inactive lepromatous patients having the anti-PGL-I antibodies; on the other hand, 6(50%) of 12 lepromatous patients treated less than 2 years had detectable PGL-I in their sera. The results thus indicate that PGL-I detection may be more suitable for monitoring the effectiveness of chemotherapy and that it may be necessary to examine for the presence of PGL-I in sera from contacts and normal populations for confirming *M. leprae* infection.

**Key Words:** *Mycobacterium leprae*, leprosy, phenolic glycolipid I, serodiagnosis

Since phenolic glycolipid I (PGL-I), a *Mycobacterium leprae* specific antigen, was isolated and characterized (Hunter and Brennan, 1981; Hunter *et al.* 1982), the antigen has been used extensively to explore the serodiagnostic tool for leprosy. The initial studies indicated that the majority (85-95%) of lepromatous patients had the abundant antibodies to PGL-I, while a much smaller portion (30-50%) of tuberculoid pa-

tients did (Cho *et al.* 1983; Young and Buchanan 1983; Brett *et al.* 1983). It was thus suggested that the lepromatous form of leprosy might be diagnosed in advance by the detection of anti-PGL-I antibodies. With the availability of semi-synthetic neoglycoprotein antigens containing the sugar epitope of PGL-I (Gigg *et al.* 1983; Fujiwara *et al.* 1984; Chatterjee *et al.* 1985), the PGL-I based serology could be implemented further to the endemic areas of leprosy.

Meanwhile, since the presence of PGL-I in clinical specimens provides unequivocal evidence of *M. leprae* infection, considerable effort has been made to detect the PGL-I antigen in body fluids. Young *et al.* (1985b), Cho *et al.* (1986), and Aguado Sanchez *et al.* (1986) reported PGL-I in sera from lepromatous patients. Cho *et al.* (1986) and Kaldany *et al.* (1986) also detected successfully PGL-I in urine. Interestingly, the PGL-I level in sera declined rapidly after chemotherapy was initiated (Cho *et al.* 1986) and was not detected in sera from patients treated more than

Received May 25, 1988

Accepted June 21, 1988

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This study was supported in part by grant MO87-5-10 from the Korean Science and Engineering Foundation and grant OCD/2/87 from the WHO Western Pacific Regional Office, Manila, Philippines.

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two months (Young *et al.* 1985b). This may suggest that the detection of PGL-I in body fluids can be employed from monitoring the effectiveness of chemotherapy as well as confirming active leprosy.

As an effort to implement the PGL-I based serodiagnostic tool in the endemic areas of leprosy, this study was initiated to determine the prevalence of anti-PGL-I antibodies among leprosy patients, their contacts and control populations. In addition, an attempt was made to detect PGL-I in sera from patients and controls with antibodies to the antigen.

## MATERIALS AND METHODS

### Serum specimens

Serum specimens were obtained from 205 leprosy patients presented to the Skin Disease Clinic in Taegu City, Korea, and from 197 leprosy patients residing in the resettlement villages in the vicinity of Taegu. Patients were classified according to the criteria established by Ridley and Jopling (1966) as polar tuberculoid (TT), borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL), and polar lepromatous (LL).

One hundred and three sera were obtained from household contacts in the leprosy resettlement villages. Serum samples were also obtained from 200 controls presented for blood chemical tests with other than leprosy to the Yonsei Medical Center in Seoul which is a low endemic area of leprosy (prevalence: 0.09/1000, Kor. Lepr. Contl. Assoc., 1986) and from 200 controls, mostly with skin problems, visiting the Skin Disease Clinic in Taegu which has been a high endemic area of leprosy (prevalence: 2.01/1000).

### Antigen

PGL-I of *M. leprae* and a neoglycoprotein antigen, natural disaccharide-octyl-bovine serum albumin (ND-O-BSA) (Chatterjee *et al.* 1986) were kindly provided by Patrick J. Brennan, Colorado State University, Fort Collins, Colorado, USA. ND-O-BSA was used as the antigen for detecting anti-PGL-I antibodies throughout the study.

### Detection of anti-PGL-I antibodies

An enzyme-linked immunosorbent assay (ELISA) described by Voller *et al.* (1979) was employed with minor modification as reported previously (Cho *et al.* 1983). Briefly, 50  $\mu$ l of diluted ND-O-BSA (20 ng sugar/ml) in carbonate buffer, pH 9.6, was added to

the wells of U-bottom microtiter plates (Dynatech Laboratories Inc., Alexandria, Va., USA), and incubated overnight at 37°C in a moist chamber. The wells were then washed with phosphate buffered saline solution, pH 7.4, containing 0.05% tween 20 (PBST) and blocked by the addition of 100  $\mu$ l of PBST-0.5% BSA at 37°C for 1 h. After emptying the wells, 50  $\mu$ l of serum diluted 1:300 in PBST-5% normal goat serum (NGS) (Gibco Laboratories, Grand Island, Ny., USA) was added to the wells and incubated at 37°C for 90 min. After washing the wells, 50  $\mu$ l of affinity-purified peroxidase-conjugated goat anti-human IgM (Behring Diagnostics, San Diego, Ca., USA) diluted 1:5000 in PBST-5% NGS was added and incubated at 37°C for 1 h. After another washing, 50  $\mu$ l for substrate solution, H<sub>2</sub>O<sub>2</sub>-o-phenylenediamine, was added to the wells and incubated at room temperature for about 15 min. The reaction was then stopped with 50  $\mu$ l of 2.5 N H<sub>2</sub>SO<sub>4</sub> and the absorbance was read at 490 nm. Each test was performed in triplicate.

### Detection of PGL-I antigen from serum

PGL-I antigen detection procedures described previously (Cho *et al.* 1986) were employed with minor modification. For serum lipid extraction, 100  $\mu$ l of serum was added to a filter paper disc (1/2 inch in diameter) and dried completely. Lipids were then extracted using 2-3 ml of CHCl<sub>3</sub>: CH<sub>3</sub>OH (2:1) solution and dried under N<sub>2</sub>. Serum lipids were dissolved in CHCl<sub>3</sub> and applied to a pasteur pipette packed with florisil, 60-100 mesh, (Sigma Chemical Co., St. Louis, Mo., USA) and eluted with CHCl<sub>3</sub>, followed by 5% CH<sub>3</sub>OH in CHCl<sub>3</sub>. The lipid fraction eluted with 5% CH<sub>3</sub>OH was saved and dried under N<sub>2</sub> and examined for the presence of PGL-I.

A dot-ELISA described by Hawkes *et al.* (1982) was employed with minor modification as reported previously (Cho *et al.* 1986; Young *et al.* 1985a). The lipid fraction was dissolved in 100  $\mu$ l of hexane and a 5  $\mu$ l portion was applied to a tufryn (polysulfone) membrane (HT-200) (Gelman Sciences Inc., Ann Arbor, Mi., USA), followed by the rest of the ELISA steps. A high titer of rabbit anti-PGL-I antibody (a gift from P.J. Brennan) was used for the primary antibody and peroxidase-conjugated goat anti-rabbit IgG (Cooper Biomedical Inc., Malvin, Pa., USA) was used as the secondary antibody. For color development, 4-chloro-1-naphthol (Biorad Laboratories, Richmond, Ca., USA) was used and the results were read visually.

### Statistics

The chi-square test was used to compare the

prevalence of anti-PGL-I antibodies among leprosy patients and control populations.

## RESULTS

### Detection of anti-PGL-I antibodies

The prevalence of anti-PGL-I IgM antibodies among leprosy patients and control populations is shown in Table 1. The mean absorbance value and standard deviation (s.d.) of controls from the Seoul area (low endemic for leprosy) were 0.036 and 0.075, respectively. A serum was considered positive if the absorbance for the serum exceeded the mean by two s.d., and an absorbance of 0.200 was used as the criteria for seropositivity in this study. Of 200 controls from the Seoul area, 3 (1.5%) were seropositive to ND-O-

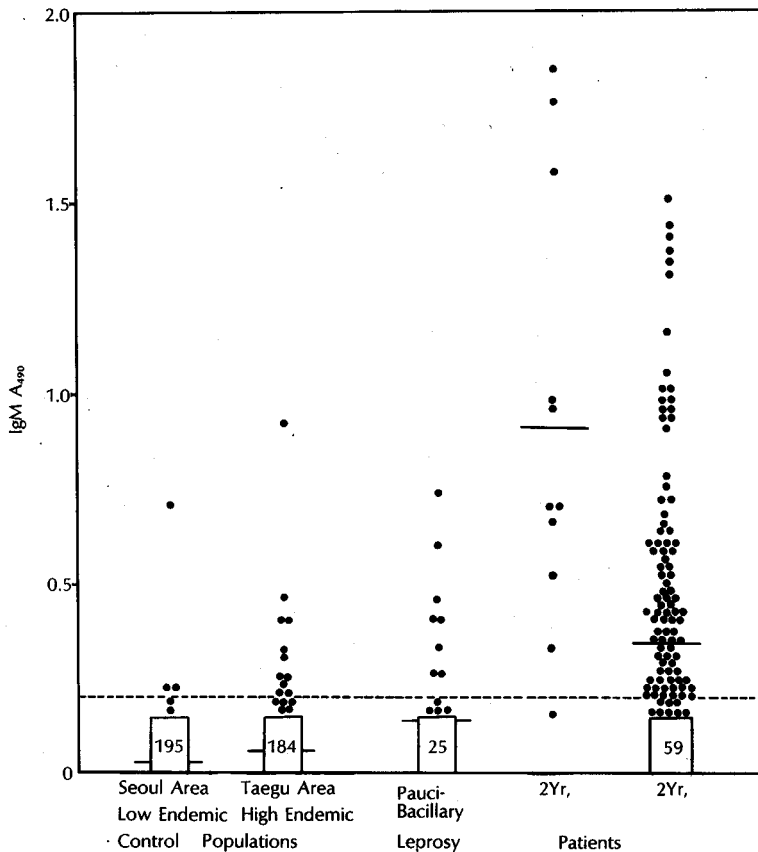
**Table 1. Prevalence of anti-PGL-I antibodies in sera from leprosy patients and controls**

Subjects	No. assayed	Seropositive*		P value
		No.	(%)	
Controls				
Seoul area	200	3	(1.5)	
Taegu area	200	11	(5.5)	<0.05
Leprosy patients				
Tuberculoid (TT+BT)	37	8	(21.6)	
Lepromatous (BL+LL)	167	99	(59.3)	<0.001**
<2 yr therapy	12	11	(91.7)	<0.05***
≥2 yr therapy	156	88	(56.4)	

\* Seropositive:  $A_{490} \geq 0.200$ .

\*\* Significant vs. tuberculoid patients.

\*\*\* Significant vs. patients with  $\geq 2$  yr therapy.



**Fig. 1.** Scattergram of anti-PGL-I IgM reactivity in sera from leprosy patients and controls. Each point represents one individual and the numbers in boxes indicate the number of individuals with absorbance less than 0.150. All points above the dashed line (the absorbance: 0.200) are considered positive. The solid lines represent the mean absorbance for each group.

**Table 2. Prevalence of anti-PGL-I antibodies among leprosy patients and their contacts in resettlement villages**

Subjects	No. assayed	Seropositive*		Mean absorbance**
		No.	(%)	
Leprosy patients				
Tuberculoid (TT+BT)	28	4	(14.3)	0.015
Lepromatous (BL+LL)	169	69	(40.8)	0.252
Contacts	103	10	(9.7)	0.062

\* Seropositive:  $A_{490} \geq 0.200$ .

\*\* Mean absorbance for the total numbers examined.

BSA. The prevalence of anti-PGL-I antibodies among controls from the Taegu area which is considered highly endemic for leprosy was 5.5% (11 of 200 sera tested) and was significantly higher than that from the Seoul area ( $p < 0.05$ )

Among leprosy patients, the seropositivity was 23.7% in the tuberculoid group and 58.7% in the lepromatous group, respectively, and the difference between the groups was statistically significant ( $p < 0.001$ ). When seropositivity was related to the duration of chemotherapy, the prevalence among patients with less than two years was 91.7% and was significantly higher than that (56.4%) among patients who were treated for more than two years ( $p < 0.05$ ).

Figure 1 shows the absorbance of each patient and the mean absorbance value of each group. Patients with the lepromatous form of leprosy had in general high anti-PGL-I IgM antibody levels, particularly among patients with short term chemotherapy. The mean absorbance was 0.911 for lepromatous patients with short-term chemotherapy and 0.349 for those treated more than 2 years.

The prevalence of anti-PGL-I IgM antibodies among leprosy patients and their contacts in the resettlement villages is shown in Table 2. The seropositivity among tuberculoid patients was 14.3% and was not significantly different from that (23.7%) among outpatients. However, the prevalence among lepromatous patients was 40.8% which was significantly lower than that (58.7%) among outpatients ( $p < 0.001$ ). Of 103 household contacts, 10 (9.7%) were seropositive; the seropositivity among contacts was thus higher than that (5.5%) of the controls in the high endemic area, although the difference was not statistically significant.

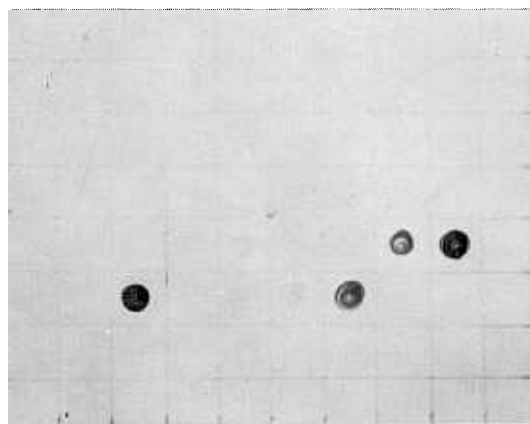
### Detection of PGL-I antigen

When some of the sera positive to PGL-I were tested for the presence of the antigen, none of the controls and contacts had detectable PGL-I (Table 3).

**Table 3. Detection of PGL-I in sera from leprosy patients, their contacts, and controls**

Subjects	No. assayed*	PGL-I detected	
		No.	(%)
Controls	14	0	
Contacts	8	0	
Lepromatous patients			
<2 yr therapy	12	6	(50.0)
$\geq 2$ yr therapy	42	0	

\* All sera examined were positive in anti-PGL-I antibodies.



**Fig. 2.** Detection of PGL-I using Dot-ELISA. A 5  $\mu$ l portion of serum lipid dissolved in hexane was applied to each square. Four dark spots indicate relatively large amounts of PGL-I; the other two faint spots also give evidence of PGL-I.

Of the 12 lepromatous patients with less than two years of chemotherapy, 6 (50%) gave evidence of PGL-I in their sera (Fig. 2). In contrast, PGL-I was not detected

in sera from the 42 lepromatous patients who had been treated for more than 2 years but had still high levels of anti-PGL-I antibodies.

## DISCUSSION

The objectives of this study were (1) to investigate the prevalence of anti-PGL-I antibodies among leprosy patients, their contacts, and control populations and (2) to explore the use of PGL-I detection for monitoring the effectiveness of chemotherapy.

Since the major immunoglobulin class to PGL-I was IgM (Cho *et al.* 1983; Young *et al.* 1984), only IgM antibodies to the antigen were under consideration throughout this study. For the antigen, Chatterjee *et al.* (1986) reported that the neoglycoprotein antigen, ND-O-BSA, has greater sensitivity and specificity than the native glycolipid in detecting anti-PGL-I antibodies. In this study, therefore, ND-O-BSA was used to detect anti-PGL-I IgM antibodies.

The criteria for the seropositivity of *M. leprae* infection based on the control populations in the low endemic area for leprosy in Korea was 0.200 which was slightly higher than that (0.120) determined for the control populations from non-endemic areas (Chatterjee *et al.* 1986). Nevertheless, the prevalence (1.5%) of anti-PGL-I antibodies among controls in the Seoul area was similar to that (2.4%) in Colorado. The higher seropositivity among controls in the Taegu area (5.5%), may reflect the high endemicity for leprosy, thus indicating that residents in the high endemic area have a greater possibility of exposure to *M. leprae*. This observation, however, needs to be reconfirmed by studies using control populations matched with age, birth place, occupation, etc.

The prevalence of anti-PGL-I antibodies among lepromatous patients diagnosed recently or treated less than 2 years was greater than 90%, thus supporting previous reports (Cho *et al.* 1983 and 1984; Bach *et al.* 1986). This may indicate that lepromatous patients can be detected by serological test before clinical signs appear; however, diagnosis of more than half of the tuberculoid patients may be missed.

The lower level of anti-PGL-I IgM antibodies among patients treated more than 2 years may reflect the effectiveness of chemotherapy, although the reason why the IgM antibodies last so long remains to be explained. In addition, the levels of anti-PGL-I antibodies in the sera from leprosy patients in the resettlement villages were lower than those from patients visiting the leprosy clinic. This may suggest that patients in the villages have been in an inactive state for a longer period of time, i.e., longer chemotherapy.

Of interest was to note that the seropositivity among family contacts of leprosy patients was apparently higher than that among control populations in the same geographical area, thus reflecting more frequent exposure to *M. leprae* among the contacts. However, the seropositivity rate among contacts in the resettlement villages (9.7%) was much lower than the rate reported in household contacts from Culiacan, Mexico (23%), and that from Sri Lanka (33%) (Buchanan *et al.* 1983). This may be due to the fact that most of patients residing in the resettlement villages are bacteriologically inactive and thus, have less chance of transmitting the bacillus to their families.

Despite the specificity of PGL-I for *M. leprae*, the circulating anti-PGL-I antibodies may provide indirect evidence of exposure to the bacillus at undetermined time. Therefore, it has been difficult to assess what the presence of anti-PGL-I antibodies means to a particular patient or contact, particularly in chronic diseases like leprosy. To overcome this uncertainty, attempts to detect PGL-I in body fluids have been made, because the antigen provides unequivocal evidence of *M. leprae* infection. PGL-I has been detected in serum specimens (Young *et al.* 1985b, Cho *et al.* 1986, and Aguado Sanchez *et al.* 1986) and in urine samples (Cho *et al.* 1986; Kaldany *et al.* 1986). Interestingly, PGL-I disappeared from serum and urine within 2-3 months after chemotherapy was initiated, thus strongly indicating that the PGL-I detection can be employed for monitoring the effectiveness of chemotherapy.

In this study, six of 12 lepromatous patients treated less than two years had detectable PGL-I, in fact, three of them were new patients and the others were treated about two months. However, none of the lepromatous patients treated for more than two years and none of the controls and family contacts seropositive to PGL-I had detectable PGL-I despite relatively high levels of anti-PGL-I antibodies in their sera. This may suggest that long-term treated patients examined in this study are bacteriologically inactive (in fact, BI negative in most cases) regardless of anti-PGL-I antibodies and that controls and contacts also do not have active disease. Accordingly, it may be desirable to examine all seropositive sera for the presence of PGL-I for confirming *M. leprae* infection as well as monitoring the effectiveness of chemotherapy of leprosy.

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