Comparison of PGL-I Level with AFB Numbers in Foot Pad Suspension

TO THE EDITOR:

Since the time that phenolic glycolipid-I (PGL-I) was first isolated and characterized as a *Mycobacterium leprae*-specific product ([4, 5]), it has been widely used in serological tests for leprosy. Besides its use for purposes of detecting antibodies to the antigen, PGL-I has been found in various clinical specimens, such as serum ([1–3, 10]), urine ([6]), and tissues ([8, 9]), etc. However, PGL-I has never been assayed in tissues of mouse foot pads inoculated with *M. leprae* where it could prove to be a useful surrogate of acid-fast bacilli (AFB) numbers. Therefore, we attempted to measure the levels of PGL-I in a mouse foot pad suspension using the dot enzyme-linked immunosorbent assay (ELISA) described previously ([2, 3]). Briefly, foot pad suspensions (1.0–1.7 ml) were lyophilized, and the lipids were extracted with chloroform : methanol (2:1) solution. After application to a florisil column, the chloroform : methanol (19:1) elute was examined for the presence of PGL-I by dot-ELISA using rabbit anti-*M. leprae* antiserum containing anti-PGL-I antibodies. A series of normal mouse foot pad suspensions containing different amounts of the standard PGL-I were processed using the same procedures to determine the test parameters for

THE TABLE. *Detection of PGL-I in foot pad suspensions.*

AFB numbers counted	No. assayed	PGL-I-positive[a]	PGL-I level (ng)
		No. (%)	Mean ± S.D.[b]
$<7.22 \times 10^3$ or $<1.77 \times 10^4$	14	1 (7.1)	7.0
7.22×10^3 –9.4×10^4	15	8 (53.3)	34.7 ± 39.6
1.0×10^5 –1.0×10^6	15	15 (100)	98.0 ± 89.7
$>1.0 \times 10^6$	14	14 (100)	353.4 ± 428.2

[a] Determined by dot-ELISA.
[b] Calculated based on the suspensions containing PGL-I.

the PGL-I assay. The numbers of AFB in these foot pad suspensions were obtained microscopically by standard procedures (7), 60 oil immersion fields being counted. If there were no AFB in 60 fields, no *M. leprae* were considered to have been present in the sample.

A total of 58 foot pad suspensions were examined. PGL-I was detectable in all suspensions, 29 in number, containing more than 1.0×10^5 AFB (The Table). Of 15 suspensions containing 7.22×10^3 to 9.4×10^4 AFB, 8 specimens had detectable PGL-I. Also, PGL-I was detectable by dot-ELISA in one of 14 suspensions containing less than 7.22×10^3 or less than 1.77×10^4, which were considered AFB negative. When the PGL-I level was compared with the total AFB numbers in each suspension, there was a strong correlation ($r = 0.834$) (The Figure). Interestingly, PGL-I concentration in foot pad suspension was much higher (about 20 times) than the calculated PGL-I amount based on the report that about 2.3% of *M. leprae* dry weight was PGL-I (4, 5). This observation supports the contention that the live bacilli actively secrete PGL-I into the surrounding tissues, and that the antigen may be trapped in tissues for a long time. The results also showed that the PGL-I level in tissues corresponded approximately to the AFB numbers, especially at the critical level (10^5 AFB) when the growth of the usual 5×10^3 *M. leprae* inoculum would have demonstrated unequivocal multiplication (7). Therefore, it should be possible to use the PGL-I detection techniques instead of counting the AFB to determine bacillary load in foot pad suspensions.

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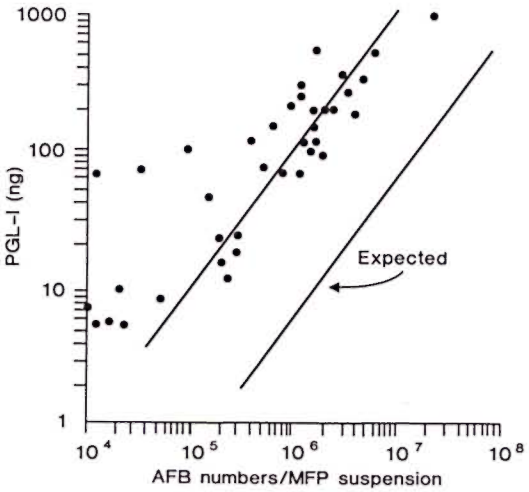

THE FIGURE. Comparison of PGL-I level with AFB numbers in the foot pad suspensions. Each dot indicates a suspension. The "expected line" was drawn based on PGL-I amount calculated from the numbers of *M. leprae*.

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