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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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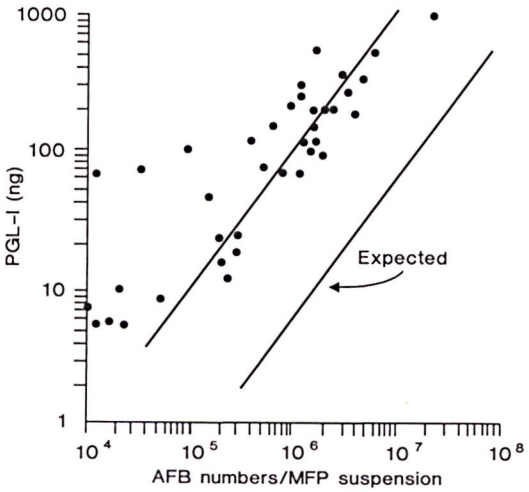

THE FIGURE. Comparison of PGL-I level with AFB numbers in the foot pad suspensions. Each dot indicates a suspension. The "expected line" was drawn based on PGL-I amount calculated from the numbers of *M. leprae*.

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Long-Term Follow up of Lepromatous Leprosy Patients Receiving Intralesional Recombinant Gamma-Interferon

TO THE EDITOR:

The primary defect in lepromatous leprosy is probably the lack of generation of sensitized T cells ([1]) which results in deficiency of gamma-interferon (IFN-γ) in the lesion ([5]). Macrophages in the tissue as well as in the circulation of lepromatous patients remain functionally normal because they release oxygen intermediates when activated by IFN-γ and other antigens ([3]). Kaplan, *et al.* in their study on the effect of intralesional recombinant IFN-γ (rIFN-γ) have demonstrated the accumulation of lymphocytes and monocytes, marked thickening of the epidermis, and increased expression of Ia and γ-IP-10 at the site of injection. With repeated injections of rIFN-γ there was a reduction in OKT-6 cells in the dermis and a distinct fall in the tissue bacterial index (BI) (5,000–10,000-fold) at the site of injection. This was often associated with the formation of an epithelioid granuloma with multinucleated giant cells ([2, 4]). The local changes prompted us to follow these cases to see whether intralesional rIFN-γ also influences tissue response at distant sites.

Twenty-two lepromatous leprosy patients (15 LLp, 7 LLs) were included in this study. There were 18 males and four females whose ages ranged from 23–58 years. Out of these 22 patients, six cases were untreated and 15 cases were on treatment for less than 1 year. Only one patient was on antileprosy drugs for more than 5 years. Lyophilized rIFN-γ (Boehringer, Ingelheim am Rhein, Germany) specific activity 2×10^7 U/mg protein was diluted and 10 μg IFN-γ in 100 μl of excipient was injected at the lesional site. The number of such injections in an individual patient varied from 1 to 3 or more. If a patient received more than one injection, it was on consecutive days and into the same lesion.

Patients were subjected to clinical charting, slit-skin smear, skin biopsy, and lepromin testing initially and then at an interval of 3–6 months for 18 months. All patients were on conventional multidrug therapy (MDT) throughout the study.

The clinical response in all 22 patients was similar to what we observe with conventional MDT in our clinic. None showed evidence of clinical or histological upgrading or lepromin conversion. Severe erythema nodosum leprosum (ENL) was observed in four patients and mild-to-moderate ENL was seen in seven patients. In two patients neural pain was aggravated.

The average fall in the BI was of 1.0 (Ridley's scale) after 18 months of follow up. Histological examination revealed that the reduction in the size of the granuloma was 1.16 and the fall in the granuloma BI was 1.01 after 18 months of follow up (The Table).

The nature of the infiltrate was predominantly of the macrophage type, and the influx of lymphocytes was insignificant up to