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# Relationships between PGL-1 antigen in serum, tissue and viability of *Mycobacterium leprae* as determined by mouse footpad assay in multibacillary patients during short-term clinical trial

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*Summary* In connection with a 56-day controlled clinical trial for comparing the therapeutic effects between pefloxacin and ofloxacin in 21 lepromatous patients, we have studied the relationships between PGL-1 antigen level in serum and in skin and serum PGL-1 antibody titre on the one hand, and the viability of *Mycobacterium leprae*, as measured by serial mouse footpad inoculations, and other bactericidal parameters on the other. Before and during treatment, significant correlation was found between serum PGL-1 level and the morphological index (MI), and with the number of viable organisms per mg skin tissue. However, neither serum PGL-1 antibody titre nor skin PGL-1 antigen level showed significant change during the 56-day trial. Because the reduction of serum PGL-1 level was well correlated but less pronounced as compared with the evolution of viable organisms during treatment, the serum PGL-1 antigen assay may be useful as an early indicator of response to chemotherapy in short-term clinical trial, but it is unlikely to replace mouse footpad inoculation for the evaluation of viability of *M. leprae*.

## Introduction

Serial mouse footpad inoculations have been applied as the most efficient technique for monitoring the chemotherapeutic effects of antimicrobials in leprosy research.[1] Nevertheless, the system is time-consuming and expensive. In addition, even using nude mice, at best one can measure only the initial 5 to 6 logs of killing[2] far in advance, previously untreated lepromatous leprosy patients which may have $10^{10}$ to $10^{11}$ viable organisms before treatment. Obviously, a more sensitive, more rapid, and preferably more simple


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technique for measuring the changes of viability of *Mycobacterium leprae* should be developed. Several *in vitro* systems[3–6] have been reported to be able to demonstrate the viability of *M. leprae*. However, all of these methods require further investigation and independent verification on their specificity and sensitivity.

In view of the following facts: 1, phenolic glycolipid I (PGL-1) is a *M. leprae*-specific antigen[7]; 2, the techniques to measure PGL-1 antibody[8, 9] as well as antigen[10, 11] are available; and 3 high levels of PGL-1 antigen can be found in serum[10] as well as in skin[12, 13] in untreated lepromatous patients, and it was reported that the serum PGL-1 antigen decreased rapidly after commencing effective chemotherapy,[11] it is likely that PGL-1 antigen assay may be applied as an indirect index on the viability of *M. leprae* during a short-term clinical trial. In connection with a controlled clinical trial in comparing the therapeutic effects between pefloxacin (PEFLO) and ofloxacin (OFLO) among lepromatous patients,[2] we have studied the relationships between PGL-I antigen in serum and in skin on the one hand, and the viability of *M. leprae* as measured by serial mouse footpad inoculations and other bacteriological parameters on the other.

## Materials and methods

### 1 PATIENTS

Twenty-one previously untreated lepromatous leprosy patients attending the R. Follereau Institute at Adzope, Côte d'Ivoire, were selected for the trial. The characteristics of these patients have been described elsewhere.[2] These patients were allocated randomly into two groups: 11 were treated with PEFLO and 10 were treated with OFLO.

### 2 PROTOCOL OF TREATMENT

The patients were treated with a single dose of 800 mg of PEFLO or OFLO on day 1, a daily dose of either 800 mg of PEFLO or 400 mg of OFLO from day 7 to day 56. At the end of the trial, they were prescribed the standard WHO multidrug therapy.[14]

### 3 BIOPSIES AND SERA COLLECTION

The sera and biopsies were collected on day 0 (before treatment) and on day 7, 14, 28, 56 after starting the treatment.

Prior to the treatment, for each patient 2 biopsies (biopsy 1 and 2) were taken from 2 different lesions. The biopsy containing the higher number of acid-fast bacilli (AFB) per mg of tissue and MI was selected for mouse inoculation and referred to as biopsy 1. The further sequential biopsies were taken from the same lesion of biopsy 1. All of the biopsies were sent as fresh tissues to Paris (Faculté de Médecine Pitié-Salpétrière) in refrigerated containers for inoculation.

The sera and the pieces of biopsies remaining after mouse inoculation, were kept frozen before being sent in refrigerated containers to Tahiti, for PGL-1 antigen and antibody determination.

## 4 MEASUREMENT OF VIABILITY OF *M. LEPRAE* BY MOUSE INOCULATION AND OTHER BACTERIOLOGICAL PARAMETERS FROM SKIN BIOPSIES

The procedure for measuring the proportion of viable organisms by inoculation into footpads of normal mice with serial dilutions of the bacterial suspension have been described.[15] In the present trial, the organisms recovered from pretreatment specimen (day 0) were inoculated into normal mice with four different dilutions ($5 \times 10^3$, $5 \times 10^2$, $5 \times 10^1$, $5 \times 10^0$ AFB) per footpad; with organisms recovered from day 7 and day 28 specimens one dilution ($5 \times 10^3$) was inoculated into normal mice and one dilution (maximum available number of organisms) into nude mice; whereas only one dilution ($5 \times 10^3$) was used in inoculating normal mice with organisms recovered from day 14 and day 56 specimens. The proportion of viable bacilli was calculated by the analysis of median infectious dose (ID50).[1]

Before mouse inoculation the concentration of *M. leprae* in each biopsy was determined and reported as the total number of acid-fast bacilli (AFB) per mg of tissue. The morphological index (MI) of these bacilli was determined as the percentage of solid-staining bacilli.[16]

The number of viable bacilli per mg of wet tissue was calculated by multiplying the proportion of viables with the number of AFB per mg of tissue.

## 5 PGL-1 ANTIBODY AND ANTIGEN ASSAYS

Anti-PGL-1 IgM antibody was detected by standard ELISA test,[8] and using the synthetic trisaccharide (NTP) (Nara University, Japan) as antigen. The antibody titre was determined as the reciprocal of the last dilution of the serum giving a positive result according to a cut-off of 0·200 optical density unit determined previously.[11]

PGL-1 antigen detection in the serum was assessed according to a methodology already described.[10, 11] Briefly after extraction from 0·5 ml of lyophilized serum with a mixture of chloroform and methanol 2:1, the PGL-1 was purified by chromatography on a small florisil column and the extract issued from the column quantitated by DOT–ELISA on nitrocellulose membrane. Purified PGL-1 (Colorado State University, Fort Collins, USA, through NIAD contract No. A1 52582) was used as a standard for quantitative assay. The results were expressed in nanograms (ng) of PGL-1 per ml of serum. The sensitivity (detectability) of the test was of 6 ng/ml of serum.

The procedure of PGL-1 antigen detection in the biopsies was as follows: each biopsy was weighed wet, homogenized in phosphate saline buffer pH 7·2 and the suspension lyophilized. The following procedure of extraction and purification was the same as for the sera and the results were expressed in ng of PGL-1 per mg of tissue. The specificity of the assay was such that all the skin tissues from 5 non-leprosy individuals, obtained during plastic surgery, were negative. The sensitivity (detectability) of the test was of 0·3 ng/mg of tissue.

## 6 STATISTICS

The results were analysed using the software 'Statgraphics' (Uniware Products, USA). Since the sample size was relatively small and the data did not conform to a normal distribution, nonparametric methods were used.[17]

The decrease of the PGL-1 in serum and tissue for each patient during the treatment was expressed by the percentage of reduction as compared to their respective initial PGL-1 level before treatment. The comparison between the 2 groups of patients was performed using the Mann and Whitney's two-tailed U test.

The relationships between the different parameters studied were analysed using the Spearman's rank correlation coefficient $r'$ tested by the $t$ test of Student.

## Results

### 1 PGL-1 ANTIGEN AND ANTIBODY BEFORE THE TREATMENT

Two sets of pretreatment results related to the number of AFB per mg of tissue and MI were available. The mean number of AFB per mg tissue and the mean MI were calculated and used for the correlation analysis.

#### *Anti-PGL-1 IgM antibody*

All 21 patients were anti-PGL-1 IgM positive before treatment, and the antibody titres varied from 2000 to 128,000. The geometric mean titre for those patients was 14,020. The correlations between anti-PGL-1 antibody and the number of AFB per mg tissue, MI and the number of viable bacilli per mg of biopsy were analysed. The Spearman's coefficient $r'$ was respectively 0·35 ($p=0·11$); 0·26 ($p=0·24$) and 0·34 ($p=0·12$). None of these correlations was statistically significant.

#### *PGL-1 antigen in the serum*

The results of PGL-1 antigen levels in the serum are presented in Table 1. The PGL-1 concentration for the 21 patients ranged from 125 to 8000 ng/ml, and the geometric mean level was 1357 ng/ml. In 4 of these patients the organisms recovered from their pretreatment biopsies failed to infect normal mice, but still low levels of PGL-1 could be demonstrated in their serum (125 to 500 ng/ml). We have studied the correlations between PGL-1 versus number AFB per mg tissue, MI and the number of viable bacilli per mg tissue, respectively. The Spearman's rank coefficient $r'$ was calculated and corresponded respectively to 0·45 ($p=0·043$), 0·63 ($p=0·0044$) and 0·82 ($p=0·0003$). The correlation between the level of PGL-1 in the serum and the number of viable bacilli was of highest significance as compared to the correlation with the MI or the AFB per mg tissue.

No correlation was found between circulating PGL-1 antigen and anti-PGL-1 IgM in these 21 patients ($r'=-0·12$, $p=0·587$).

#### *PGL-1 antigen in the skin biopsies*

The PGL-1 antigen was determined on a small piece of skin tissue remaining after mouse inoculation. It could be done for 17 of the 21 patients studied. Two biopsies at day 0 were available for 14 of these 17 patients, a total of 31 samples were then tested, and their wet weight ranged from 7 to 25 mg. Table 2 showed that all of the pretreatment biopsies tested were positive for PGL-1 antigen ranging from 5 to 8889 ng/mg, the geometric mean level was 200 ng/mg. Two (from patients nos 4 and 13) of the 4 biopsy specimens which failed to

**Table 1.** PGL-1 in serum, mean morphological index (MI), mean AFB/mg and number of viable bacilli in 21 MB patients before treatment

| Patient no. | PGL-1 ng/ml | Mean* MI % | Mean* AFB/mg | Number viable† bacilli/mg |
|---|---|---|---|---|
| 1 | 1250 | 25·5 | $5{\cdot}5 \times 10^5$ | 10380 |
| 2 | 4000 | 17·5 | $2{\cdot}5 \times 10^6$ | 55000 |
| 3 | 500 | 6 | $1{\cdot}1 \times 10^6$ | < 70 |
| 4 | 125 | 5·5 | $1{\cdot}1 \times 10^6$ | < 70 |
| 5 | 1000 | 6 | $2{\cdot}1 \times 10^6$ | 2760 |
| 6 | 125 | 6 | $8 \times 10^4$ | < 5 |
| 7 | 2000 | 7 | $1{\cdot}5 \times 10^6$ | 340 |
| 8 | 8000 | 38 | $1{\cdot}5 \times 10^6$ | 4350 |
| 9 | 1000 | 6 | $2{\cdot}7 \times 10^6$ | 450 |
| 10 | 2000 | 36 | $3 \times 10^5$ | 69000 |
| 11 | 4000 | 19·5 | $7{\cdot}5 \times 10^5$ | 173000 |
| 12 | 5000 | 21 | $2{\cdot}5 \times 10^5$ | 690000 |
| 13 | 156 | 4 | $3 \times 10^5$ | < 18 |
| 14 | 8000 | 14·5 | $5{\cdot}7 \times 10^5$ | 173000 |
| 15 | 833 | 12 | $9 \times 10^5$ | 1384 |
| 16 | 1333 | 25·5 | $1 \times 10^6$ | 13800 |
| 17 | 2666 | 5·5 | $1{\cdot}5 \times 10^6$ | 34600 |
| 18 | 3333 | 16 | $1{\cdot}8 \times 10^6$ | 8400 |
| 19 | 666 | 24·5 | $3{\cdot}5 \times 10^4$ | 87 |
| 20 | 375 | 1·5 | $3 \times 10^5$ | 2760 |
| 21 | 8000 | 30·5 | $1{\cdot}5 \times 10^6$ | 275000 |

* Mean values obtained from biopsies 1 and 2.

† Derived from mouse footpad inoculation results of biopsy 1.

Spearman's coefficient of correlation:

PGLS/Mean MI 0·62 ($p = 0{\cdot}0044$)

PGLS/Mean BI 0·45 ($p = 0{\cdot}043$)

PGLS/No. viable bacilli 0·82 ($p = 0{\cdot}0003$).

infect mice were available for PGL-1 testing and they showed a significant amount of PGL-1. The AFB per mg tissue of these 2 biopsies were respectively $2 \times 10^6$ and $4 \times 10^5$.

The correlations between the skin PGL-1 and the AFB per mg tissue and the number of viable bacilli per mg tissue were statistically significant: the Spearman's coefficient $r'$ was respectively 0·56 ($p = 0{\cdot}002$) and 0·659 ($p = 0{\cdot}01$). There was also a significant correlation between the level of PGL-1 antigen found in the skin and in the serum: $r' = 0{\cdot}68$ ($p = 0{\cdot}008$). Conversely, the PGL-1 level in the tissue was not found to be correlated with the MI of the bacilli recovered from the same biopsy specimen ($r' = 0{\cdot}198$, $p = 0{\cdot}276$).

## 2 EVOLUTION OF PGL-1 ANTIGEN AND ANTIBODY LEVELS DURING TREATMENT

The 4 patients (nos 3, 4, 6 and 13) in which no detectable viable organisms were demonstrated in their pretreatment biopsies were removed from the study. Therefore the analysis included the data obtained from 9 patients treated with PEFLO and 8 patients treated with OFLO.

**Table 2.** PGL-1 in skin biopsy, morphological index (MI), AFB/mg and viable bacilli in 17 MB patients before treatment

| Patient No. | PGL-1 ng/mg | | MI % | | AFB/mg | | Number viable bacilli/mg biopsy 1* |
|---|---|---|---|---|---|---|---|
| | Biopsy 1 | Biopsy 2 | Biopsy 1 | Biopsy 2 | Biopsy 1 | Biopsy 2 | |
| 1 | 238 | 109 | 26 | 25 | $6 \times 10^5$ | $5 \times 10^5$ | 10380 |
| 2 | 870 | 500 | 28 | 7 | $2 \times 10^6$ | $3 \times 10^6$ | 55000 |
| 3 | ND | 52 | 4 | 8 | $2 \times 10^6$ | $2 \times 10^5$ | < 70 |
| 4 | 294 | 62 | 5 | 6 | $2 \times 10^6$ | $2 \times 10^5$ | < 70 |
| 7 | 250 | 227 | 8 | 6 | $2 \times 10^6$ | $1 \times 10^6$ | 340 |
| 9 | 952 | 526 | 5 | 7 | $5 \times 10^6$ | $4 \times 10^5$ | 450 |
| 10 | 416 | 278 | 47 | 25 | $5 \times 10^5$ | $1 \times 10^5$ | 69000 |
| 11 | 8889 | 147 | 18 | 21 | $1 \times 10^6$ | $5 \times 10^5$ | 173000 |
| 12 | 869 | 113 | 26 | 16 | $2 \times 10^6$ | $3 \times 10^6$ | 690000 |
| 13 | 73 | 139 | 5 | 3 | $4 \times 10^5$ | $2 \times 10^5$ | < 18 |
| 14 | 588 | 125 | 15 | 15 | $1 \times 10^7$ | $4 \times 10^5$ | 173000 |
| 15 | 119 | 200 | 15 | 9 | $8 \times 10^5$ | $1 \times 10^6$ | 1384 |
| 17 | 455 | 108 | 5 | 6 | $2 \times 10^6$ | $1 \times 10^6$ | 34600 |
| 18 | 104 | ND | 9 | 23 | $3 \times 10^6$ | $3 \times 10^5$ | 8400 |
| 19 | 5 | 12 | 23 | 26 | $1 \times 10^4$ | $6 \times 10^4$ | 87 |
| 20 | 16 | 192 | 3 | 0 | $4 \times 10^5$ | $1 \times 10^5$ | 2760 |
| 21 | 2857 | ND | 40 | 21 | $1 \times 10^6$ | $2 \times 10^6$ | 275000 |

ND, not done.
* Derived from mouse footpad inoculation results of biopsy 1.
Spearman's coefficient of correlation:
PGLB/MI 0·198 ($p = 0·276$)
PGLB/BI 0·56 ($p = 0·002$)
PGLB/No. viable bacilli 0·659 ($p = 0·01$).

*Evolution of Anti-PGL-1 IgM antibody*

As compared to the initial level, no significant change of anti-PGL-1 IgM titre was observed in any of the patients during the 56 days of the trial.

*Evolution of PGL-1 antigen in the serum*

The evolution of the PGL-1 levels are represented in Table 3. The percentage of decrease of the PGL-1 level on days 7, 14, 28 and 56 was calculated for each patient, as compared to their level before treatment. Then the median of the percentage of reduction for each group was obtained. For both groups of patients this median of reduction decreased gradually, and reached 87% for PEFLO and 85% for OFLO on day 56. No significant difference in the PGL-1 reduction was found between the 2 groups using the Mann–Whitney's U test ($\Sigma < 0·01$, $p > 0·99$).

*Evolution of PGL-1 antigen in the skin*

Table 4 presents the individual results of the PGL-1 from sequential biopsies in 14 patients (8 treated with PEFLO and 6 treated with OFLO) from whom enough skin tissue was available after mouse inoculation. The percentage of decrease of the PGL-1 was calculated for each patient, as compared to the level before treatment. The medians of the

**Table 3.** Evolution of PGL-1 antigen in the serum of multibacillary patients treated either with ofloxacin or pefloxacin

| Patient No. | PEFLO (P) or OFLO (O) | PGL-1 in ng/ml (percentage of reduction as compared to level before treatment) | | | | |
|---|---|---|---|---|---|---|
| | | Before treatment | Day 7 1 dose | Day 14 8 doses | Day 28 22 doses | Day 56 50 doses |
| 1 | O | 1250 (0) | — | 4000 (−220) | 2000 (−60) | 500 (60) |
| 2 | P | 4000 (0) | — | 1000 (75) | 1000 (75) | 500 (87,5) |
| 5 | P | 1000 (0) | — | — | 1000 (0) | 280 (72) |
| 7 | O | 2000 (0) | — | — | 1000 (50) | 375 (81,25) |
| 8 | O | 8000 (0) | — | — | 2000 (75) | 500 (93,75) |
| 9 | P | 1000 (0) | 500 (50) | 250 (75) | 125 (87,5) | 125 (87,5) |
| 10 | P | 2000 (0) | 500 (75) | 250 (87,5) | 250 (87,5) | — |
| 11 | P | 4000 (0) | 4000 (0) | 2000 (50) | 1000 (75) | 190 (95,25) |
| 12 | P | 5000 (0) | 4000 (20) | 8000 (−60) | 4000 (20) | 1000 (80) |
| 14 | O | 8000 (0) | 1000 (87,5) | 4000 (50) | 1000 (87,5) | 333 (95,85) |
| 15 | O | 833 (0) | 500 (40) | 500 (40) | 416 (50) | 292 (64,95) |
| 16 | O | 1333 (0) | 500 (62,5) | 1000 (25) | 333 (75) | 141 (89,4) |
| 17 | P | 2666 (0) | 4000 (−50) | 4000 (−50) | 500 (81,25) | 333 (87,5) |
| 18 | P | 3333 (0) | 1000 (70) | 2000 (40) | 500 (85) | 250 (92,5) |
| 19 | O | 666 (0) | 1000 (−50,15) | 500 (24,9) | 125 (81,2) | 62 (90,7) |
| 20 | O | 375 (0) | 250 (33,3) | 250 (33,3) | 125 (66,7) | 125 (66,7) |
| 21 | P | 8000 (0) | 8000 (0) | 8000 (0) | 8000 (0) | 833 (89,6) |
| Median of % reduction for PEFLO ± CL* | | 0% | −20 ± 42% | 45 ± 56% | 75 ± 34% | 87 ± 4% |
| Median of % reduction for OFLO ± CL* | | 0% | 40 ± 20% | 29 ± 10% | 71 ± 16% | 85 ± 15% |

* Confidence limit of median.

Difference of PGL-1 reduction between the 2 groups using the Mann–Whitney U-test was not significant.

percentage of reduction for both groups of patients were obtained: these figures were found to fluctuate with large confidence limits. It should be noted that the number of samples tested was small for each series (4 to 6 samples). The PGL-1 level in the skin tissue was not likely to decrease in both groups of patients during the 56 days' trial. Simultaneous testing of different parts of a same biopsy showed a great variability of PGL-1 concentration from one part to another part of a lesion (results not reported), and probably explained some extensive variations observed in sequential biopsies of the same patient.

## 3 RELATIONSHIPS BETWEEN THE REDUCTION OF PGL-1 IN SERUM, THE REDUCTION OF MI AND THE KILLING RATE OF *M. LEPRAE* DURING THE TREATMENT

The complete clinical and bacteriological results of this study are reported elsewhere.[2]

### *Relationships between the reduction of PGL-1 in the serum and the reduction of the MI*

In the current trial, the MI fell gradually and significantly during the treatment in both groups of patients.[2] Because the relationships between the reduction of PGL-1 and the

**Table 4.** Evolution of PGL-1 antigen in the skin tissue of multibacillary patients treated either with ofloxacin or pefloxacin

| | | PGL-1 in ng/ml (percentage of reduction as compared to level before treatment) | | | | |
|---|---|---|---|---|---|---|
| Patient | PEFLO (P) or OFLO (O) | Before treatment | Day 7 1 dose | Day 14 8 doses | Day 28 22 doses | Day 56 50 doses |
| 1 | O | 238 (0) | 192 (19,3) | 208 (12,6) | — | 113 (52,5) |
| 2 | P | 870 (0) | 588 (32,4) | 1176 (−35,2) | — | — |
| 7 | O | 250 (0) | — | 104 (58,4) | 250 (0) | 192 (23,2) |
| 9 | P | 952 (0) | 1000 (−5) | 357 (62,5) | — | — |
| 10 | P | 416 (0) | — | — | — | 54 (87) |
| 11 | P | 8889 (0) | 1176 (86,8) | 555 (93,8) | 625 (93) | — |
| 12 | P | 869 (0) | — | — | 500 (42,5) | — |
| 14 | O | 588 (0) | 250 (57,5) | 769 (−30,8) | 192 (67,3) | — |
| 15 | O | 119 (0) | 217 (−82,3) | 89 (25,2) | — | 108 (9,2) |
| 17 | P | 455 (0) | 454 (0,2) | 178 (60,9) | 400 (12,1) | — |
| 18 | P | 104 (0) | 192 (−84,6) | 119 (−14,4) | 147 (−41,3) | — |
| 19 | O | 5 (0) | 166 (−33,20) | — | 3 (40) | 15 (70) |
| 20 | O | 16 (0) | 18 (−12,5) | — | — | 12 (25) |
| 21 | P | 2857 (0) | 1666 (41,7) | — | 138 (95,2) | — |
| Median of % reduction for PEFLO ± CF* | | 0% | 16 ± 30% | 61 ± 54% | 42 ± 57% | — |
| Median of % reduction for OFLO ± CF* | | 0% | −12 ± 71% | 19 ± 40% | 40 ± 61% | 25 ± 20% |

* Confidence limits of median.

Difference of PGL-1 antigen reduction between the 2 groups using the Mann–Whitney U-test was not significant.

reduction of the MI were independent from treatment, the data from the 17 patients were pooled for analysis. The Spearman's rank coefficient of correlation so obtained was $r' = 0{\cdot}46$ ($p = 5 \times 10^{-4}$).

*Relationship between the decrease of PGL-1 in the serum and the reduction of number of viable bacilli*

The number of viable *M. leprae* defined by their ability to multiply in mice before and during treatment and the median of the killing rate (percentage of viables killed) were calculated for each group of patients.[2] As the killing rate obtained from the OFLO group was not significantly different from that obtained from the PEFLO group, the data from all the 17 patients were gathered. The evolution of the number of viable organisms per $10^6$ AFB during the course of treatment with PEFLO or OFLO was summarized in a single regression equation: $Y = -0{\cdot}175X + 3{\cdot}9168$. Similarly, the regression equation of the evolution of PGL-1 per ml of serum was obtained as below: $Y = -0{\cdot}0172X + 3{\cdot}286$. According to the slopes of these two equations, the speed of reduction of the viable *M. leprae* was about 10 times higher than the speed of reduction of the PGL-1 in the serum. Figure 1 presents the evolution of the number of viables (in $\log_{10}$) and the evolution of PGL-1 (in $\log_{10}$) in the serum from 8 doses (day 14) to 50 doses (day 56) of PEFLO or

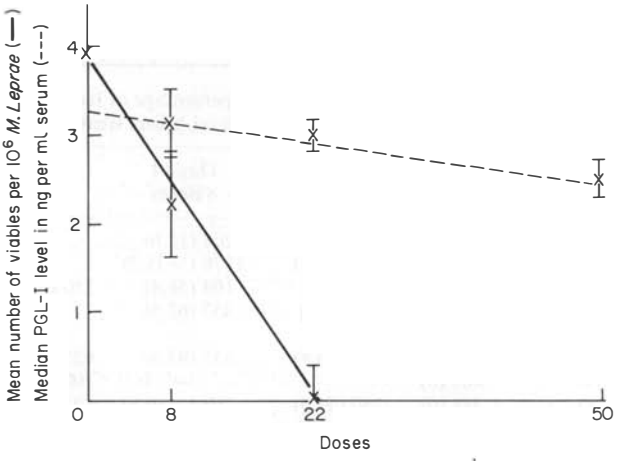


**Figure 1.** Evolution of the PGL-1 antigen in the serum and of the number of viable *M. leprae* in multibacillary patients, according to the number of doses of pefloxacin or ofloxacin. Equation for PGL-1 regression line: $Y = -0{\cdot}0172x + 3{\cdot}286$. Equation for number of viables regression line: $Y = -0{\cdot}175x + 3{\cdot}916$.

OFLO. The data after 1 dose (day 7) were not presented because both parameters were not significantly different from before treatment.

## Discussion

Before starting chemotherapy, the IgM antibody titre to PGL-1 antigen varied widely in the 21 lepromatous patients, and therefore confirmed what we have observed previously.[11] The variation was not correlated with the bacterial load in the hosts, and was neither correlated with MI nor with the number of viable organisms measured by mouse footpad inoculation. The wide variation of antibody titre probably reflects the significant variation of antibody response to *M. leprae* among lepromatous patients. The anti-PGL-1 antibody titre did not show significant changes during treatment, obviously this is due to the short-term nature of the trial. As demonstrated in patients under multidrug therapy[11,18] significant reductions of PGL-1 antibody titre might be observed only after more than one year's treatment.

Prior to the treatment, the PGL-1 antigen level in the serum of lepromatous patients was also widely distributed (125 to 8000 ng/ml), however, the level was well correlated with the number of AFB/mg tissue, the MI and the number of viable organisms in the skin. Serum PGL-1 antigen could still be detected in 4 patients whose bacilli, recovered from skin biopsies, failed to infect normal mice. Nevertheless, their antigen levels (125 to 500 ng/ml) were in the lowest range of the 21 patients tested.

The detection of PGL-1 antigen in the tissue of leprosy patients or *M. leprae*-infected armadillo has been previously reported,[7,12,13] but usually required several hundred milligrammes or even grammes of the specimen and used thin layer chromatography or high pressure liquid chromatography for characterization and quantitation. In the current study we used a modified method, consisting of rapid purification on a florisil column, followed by DOT–ELISA. As small as 10 to 20 mg of skin biopsy can be

examined for PGL-1 quantitation with the modified method. Using our method we were able to detect PGL-1 in all 31 pretreatment biopsies taken from 17 lepromatous patients, including the biopsies from 2 patients in which no viable organisms could be detected by mouse footpad inoculation. The concentration of PGL-1 in the 31 samples varied from 5 to 8889 ng/mg, and it was significantly correlated with the number of AFB/mg tissue ($r' = 56, p = 0{\cdot}002$). Similar to the elimination of AFB from the tissue, the preliminary data suggested that the complete clearance of PGL-1 antigen from the tissue may take several years after starting effective chemotherapy[12] (and unpublished data). However, up to now no systematic study has been carried out to follow-up simultaneously the clearance of organisms and PGL-1 antigen from the tissue. Such a study may well be useful in providing a better understanding of the potential value of PGL-1 antigen detection and the kinetics of PGL-1 in the hosts.

Contrary to the skin PGL-1 antigen, the PGL-1 antigen levels in the serum steadily decreased during treatment with 800 mg PEFLO daily or 400 mg OFLO daily. Similar to the reduction of viable organism as measured by mouse footpad inoculation,[2] the percentage of PGL-1 reduction after 56 days' treatment, i.e. 50 doses, did not show a significant difference between the two groups (87% and 85% respectively). Because both PEFLO and OFLO displayed strong bactericidal activity against *M. leprae*,[19, 20] the decrease of PGL-1 in the serum during a few weeks of treatment with either drug was consistent with our previous observation[11] during treatment with rifampicin, another strong bactericidal agent against *M. leprae*. Moreoever, during treatment the evolution of the viable organisms and the evolution of the antigenemia showed similar trends. However, the slope of the regression line for the reduction of viable organisms was about 10 times greater than that for serum PGL-1 reduction. As compared with the reduction of viable organisms, the delay of the clearance of serum PGL-1 antigen probably related to the involvement of different mechanisms in release and elimination of the metabolite.

Although it is unclear whether or not the detected serum PGL-1 was due to the presence of *M. leprae* in the blood, or bacillemia, the relatively rapid clearance of PGL-1 from the serum and the disappearance of bacillemia during the course of effective chemotherapy was quite similar.[21, 22] Both phenomena occurred when a sufficiently large amount of PGL-1 and *M. leprae* were still available in the tissue, suggesting that the mechanisms involved in the clearance of PGL-1 and bacillemia may have some similarities. To the best of our knowledge, up to now, no study has simultaneously measured the evolution of serum PGL-1 antigen level and bacillemia. Probably this is a reasonable approach for a better understanding of the relationship of the two different phenomena.

In conclusion, because of the correlation with the viability of *M. leprae*, the serum PGL-1 antigen assay may be useful as an early indicator of response to chemotherapy in short-term clinical trial; nevertheless, it is unlikely to replace mouse footpad inoculation for the evaluation of viability of *M. leprae*.

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