

# MODULATION OF WHOLE BLOOD IMMUNE RESPONSES TO PHENOLIC GLYCOLIPID (PGL-I) OF *M. LEPRAE*

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**Abstract:** *Mycobacterium leprae* cell wall associated components are found in large amounts in the tissues of leprosy patients, particularly those at the lepromatous pole. Among these molecules, the phenolic glycolipid (PGL-I), unique to *M. leprae* has been involved in the selective anergy observed in the lepromatous patients. Armadillo-derived *M. leprae* retains only a small proportion of the total PGL-I found in infected tissues. While, the process by which *M. leprae* is obtained from MFP material is much less harsh than that for armadillo tissue. It is therefore likely that MFP derived *M. leprae* have their PGL-I coating more intact. Therefore, the addition of PGL-I to WML *in vitro* is important for better understanding of *M. leprae* effects *in vivo*. We have studied the influence of PGL-I on TNF- $\alpha$  production by whole blood cells following stimulation with WML and MFP *M. leprae* only. PGL-I did not induce the secretion of TNF- $\alpha$  by whole blood cells but when associated with WML increased the release of this cytokine. MFP derived *M. leprae* showed the highest TNF- $\alpha$  response. Thus the difference in TNF- $\alpha$  response shown by WML and MFP ML was mainly due to the PGL-I factor, which was present in MFP ML. This increase in TNF- $\alpha$  production suggests that PGL-I play a role in the induction of TNF- $\alpha$  during natural infection.

**Key words:** *Mycobacterium leprae*; phenolic glycolipid (PGL-I); leprosy; TNF- $\alpha$ ; diagnostic.

## INTRODUCTION

Leprosy is a chronic granulomatous disease caused by *Mycobacterium leprae* affecting the peripheral nerves, mucosa of upper respiratory tract and also the eyes. In severe manifestation other organs may be involved. The immunity towards *M. leprae* is a complex process and mainly cell mediated. Mycobacterial antigens are widely distributed in tissues of patients with leprosy (Young, 1981). Among the mycobacterial antigen PGL-I is specific towards *M. leprae* and these PGL-I forms an extra cellular product forming a loose capsule around the bacillus (Hunter *et al*, 1982). Antibody responses to this antigen are also the diagnostic of exposure to *M. leprae* (Roche *et al*, 1990). PGL-I may play in the intracellular persistence of *M. leprae* in human macrophage by scavenging toxic oxygen metabolites of antimicrobial systems of host cell (Launois *et al*, 1989). PGL-I also induces suppressor T-cells in lepromatous patients and in high concentration may non-specifically suppress T-cell responses (Mehera *et al*, 1984). PGL-I is also important in uptake of *M. leprae* by macrophage (Schlesinger and Horwith, 1991).

In mycobacterial infection TNF- $\alpha$  is involved in both host protective responses and disease pathology (Barnes *et al*, 1992). Little is known about the factor, which triggers TNF- $\alpha$  release during the course of disease. There is some evidence that PGL-I is a modulatory factor in the production of this cytokine (Charlab *et al*, 2001). We

have studied the influence of PGL-I on TNF- $\alpha$  production by whole blood cells following stimulation with Armadillo derived *M. leprae* (WML) and mouse footpad derived *M. leprae* (MFPML).

WML are depleted in PGL-I as a result of purification process required following culture in Armadillo liver (Hunter *et al*, 1982) while extraction of *M. leprae* from mouse footpad is less harsh thus MFPML is likely to have intact PGL-I. These two antigens were used to stimulate the whole blood cells under same environmental condition and if any difference was seen in TNF- $\alpha$  response between these antigens it is likely be due to PGL-I factor.

## MATERIALS AND METHODS

### Study subject

The experiment was carried out among 20 individuals categorized under four groups *viz.* professionally exposed (PE), Healthy contacts (HC), Borderline tuberculoid (BT), Borderline lepromatous (BL).

### Stimulants

1 ml whole blood cells were stimulated with 10 $\mu$ g PGL-I. Mouse footpad *M. leprae* was obtained by centrifugation at 100g/2 minutes and the supernatant obtained was further centrifuged at 2200g/30 minutes. 2 $\times$ 10<sup>5</sup> organism/ml was used to stimulate 1 ml whole blood cells. 1 ml blood was stimulated with WML (2 $\times$ 10<sup>5</sup>

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organism/ml), PGL-I +WML (10µg + 2×10<sup>5</sup> organism/ml) (P.J Brennan, Colorado State University, USA) and PHA 10µg (Sigma). After 12hrs and 24hrs incubation at 5%CO<sub>2</sub> at 37°C, the plasma (supernatant) was drawn.

### ELISA

Tumor necrosis factor (TNF-α) was measured by ELISA using paired monoclonal murine antibodies. ELISA plates (Immulon 2, Dynatech, Chantilly, USA) were coated with the capture mouse anti-human TNF-α monoclonal antibody (2µg/ml) by incubating overnight at 4°C. The wells were blocked with 3% BSA in PBS. Serial dilutions of recombinant human TNF-α (Pharmingen, San Diego, CA) diluted in RPMI (Sigma) with 5% heat inactivated human pooled serum were applied in a concentration range of 2000pg/ml to 15 pg/ml in a first two rows of each ELISA plate. 20 µl of each culture supernatant was added to six wells and incubated overnight at 4°C. After washing Biotin-labeled anti-human TNF-α (1µg/ml) was added to the plate and incubated for 1 hour at room temperature. Avidin peroxidase (Sigma) (1.25µg/ml) diluted 1 in 800 was added to the plate and incubated for 1 hour at room temperature. Colour reaction was developed by adding 0.4mg/ml O-phenylenediamine dihydrochloride (Sigma) in citrate phosphate buffer (pH 5.0) and 0.006% hydrogen peroxide. The reaction was stopped with 2.5 N Sulphuric acid. The plate was read at a wavelength of 492 nm using Dynatech MRX Plate Reader. TNF-α results were expressed as mean units of TNF-α (U/ml) of six wells after subtraction of any non-specific TNF-α production in non-stimulated cultures. The minimum & maximum detection limit of the TNF-α ELISA was 15 U/ml & 2000 U/ml respectively.

Statistical analysis was done by Student's T-test.

### RESULTS

We compared the TNF-α produced by whole blood cells treated with PGL-I, PGL-I+WML, WML and MFPML at 12hours and 24 hours. Peak TNF-α response was seen at 24 hours incubation point for all the antigens used. Stimulation of whole blood cells with PGL and WML alone induced least TNF-α response while PGL-I when cultured along with WML showed the increased TNF-α response (p= 0.077). Highest TNF-α response was shown by

MFPML. T-test showed the significant difference in TNF-α response between WML and MFPML (p= 0.027, p=0.04 at 12 hrs and 24 hrs respectively).

### DISCUSSION

PGL-I is produced in large amounts during *M. leprae* infection. In view of their abundance and location in cell wall (Boddingius *et al*, 1990) PGL-I is recognized by the immune system and thus likely to play a significant role in interaction between the pathogen and its environment. Thus this study was mainly done to understand the influence of PGL-I in the production of TNF-α.

Before starting the experiment optimizations of time point and antigen were done for production of TNF-α from whole blood cells. Initially we used MFPML and WML as the stimulants and later PGL-I was included so as to strengthen the results and to give the clearer view of its influence on cytokine production. Initially six different time points i.e. 2, 4, 12, 18, 24, 36 hrs were used. Highest TNF-α response was observed between 12-24 hrs time points (Results not shown).

In this study PGL-I alone did not induced TNF-α secretion by whole blood cells but when used along with WML increased the TNF-α response. MFPML having the intact PGL-I showed the highest TNF-α response. Thus these results clearly suggest that PGL-I plays a role in the production of TNF-α. The study done by Charlab *et al*, 2001 also agrees with the results we have obtained.

MFPML is a crude preparation thus this might be contaminated with the mouse footpad (MFP) material, which might play a part in stimulating the production of TNF-α. Thus to study this fact, we compared the TNF-α response given by mouse footpad material and MFPML. Mouse footpad material did not show a detectable level of TNF-α. Thus high response shown by MFPML was not due to the contaminated mouse footpad material.

Thus in this experiment we showed that that PGL-I alone will not induce the secretion of TNF-α by whole blood cells but when cultured along with WML induced the cells to release this cytokine. The difference in the response between MFPML and WML was mainly due to the PGL-I factor, which was not present in WML. Researchers

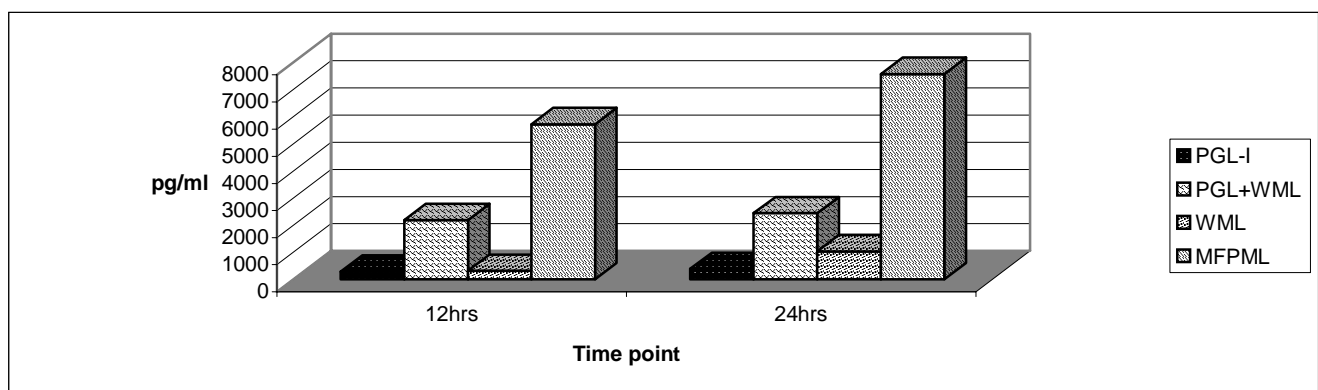


Fig. 1: 12 hrs and 24 hrs TNF-α response shown by various antigens

through out the world are using Armadillo derived *M. leprae* (WML) *in vitro* to understand the role of *M. leprae in vivo*. But our study showed that cell mediated responses shown towards WML and MFPML differs (in production of TNF- $\alpha$ ), which is likely be due to structural difference in these two forms of *M. leprae*. Thus, using MFPML *in vitro* culture will give more appropriate immunological responses as compared to WML.

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