Circulating cytokine profiles in leprosy patients

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Summary

Background Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* which is an obligate intracellular pathogen. It is characterised by a broad spectrum of clinical forms dictated by the patient’s immune response to the organism. The tuberculoid pole has good cell mediated immunity to *M. leprae*, with few lesions and bacilli while the lepromatous pole has poor immunity coupled with extensive involvement and greater bacillary load.

Methods We studied serum levels of interferon gamma and interleukin 6 in 100 patients of untreated leprosy, compared them with 30 age and sex matched normal healthy controls and co-related them with different parts of the spectrum and reactional episodes. The purpose of this study was to delineate the role of cytokines and their clinical implications in the leprosy spectrum and during reactional episodes.

Results We observed that mean cytokine levels were significantly higher in the patient group as compared to the controls. In the non reactional patient group, pure neuritic leprosy patients showed highest levels of INFγ which were directly proportional to the extent of nerve involvement. Lepromatous leprosy patients had the highest levels of IL6. Bacteriological index demonstrated a negative and positive correlation with INFγ and IL 6 levels respectively. Type I and Type II reactional patients had higher levels of INFγ and IL 6 respectively as compared to nonreactional patients.

Conclusions Our results suggest that pure neuritic leprosy and borderline tuberculoid patients in type I reaction are at greatest risk for nerve and tissue damage. Thus cytokines have the potential to play a significant role in classification, prognosis and treatment of leprosy.
Introduction

Leprosy is a chronic infectious disease which presents in a wide spectrum of clinical forms depending on the host’s immune response to *Mycobacterium leprae*, the causative agent.\(^1\) The past few years have witnessed exciting new developments in the immunology of leprosy. However, there are still many unanswered questions on the nature of immunity in leprosy and the complex changes in the immunological mechanisms. Noteworthy amongst them is the interplay between T cell subsets. Leprosy provides a unique opportunity to investigate the functions of Th1 and Th2 subsets in relation to their cytokine secretion profile since it is postulated that it is the distinct pattern of cytokines which determines the resistance or susceptibility to infection.\(^2,3\)

Cytokines are low molecular weight regulatory proteins which act at picomolar concentrations. They act as molecular signals for cell to cell communication by binding to specific receptors on target cells.\(^4,5\)

The cell mediated immune response is responsible for defence against intracellular pathogens and probably dictates the manifestations of leprosy.\(^1\) In the immunological spectrum, at one end is the tuberculoid pole with few bacilli and a good cell mediated immunity (CMI) to *M. leprae* while at the other end is the lepromatous pole with a high bacillary load and poor CMI.\(^6\) On antigenic stimulation, naïve Th cells differentiate into distinct Th1 or Th2 functional subgroups. Th1 cells secrete IL-2, IFN-\(\gamma\) and TNF which activate macrophages and elicit delayed type hypersensitivity reactions. In contrast, Th2 cells produce IL-4, 5, 6 and 10 and are responsible for antibody production, inhibition of macrophage functions and suppression of CMI.\(^7\)

The aim of the present study is to analyse serum cytokine profiles of untreated leprosy patients, compare them with healthy controls and co-relate the patterns with different parts of the spectrum and reactional episodes.

Materials and Methods

100 patients of untreated leprosy were randomly selected from patients attending dermatology OPD of Sassoon General Hospitals, Pune. After written consent, every patient was clinically assessed by detailed history, thorough medical and dermatological examination. Nerve involvement was assessed based on clinical examination: finding of definite nerve thickening, tenderness, nodularity or abscess formation. Sensory motor assessment charting was performed in the distribution of the nerve and confirmed by nerve conduction velocity studies.

After slit skin smear for acid fast bacilli and skin biopsy, the patients were classified according to Ridley-Jopling’s five subgroups (TT, BT, BB, BL, LL).\(^8\) A sixth group (pure neuritic) was added as per Consensus Classification of Indian Association of Leprologists (1982).\(^9\)

They included 40 patients of borderline tuberculoid (BT), 16 of mid-borderline tuberculoid (BB), 15 of borderline lepromatous (BL), 18 of lepromatous (LL) and 11 of pure neuritic (PN). Six patients were in reaction (five of Type one and one of Type two). Case definition for Type 1 reaction patients: patients with BT, BB or BL leprosy presenting with swollen, tender or more erythematous patches or sudden appearance of new patches, neuritis, constitutional symptoms like fever, malaise, edema of hands and feet.
Case definition for Type 2: Patients with BL or LL leprosy presenting with tender nodular swellings (erythema nodosum leprosum), constitutional symptoms, neuritis, iritis/iridocyclitis, orchitis.

All patients on anti leprosy treatment or steroids, having other systemic diseases and HIV were excluded from the study. Thirty age and sex matched healthy controls were also included. 5cc blood was collected from all patients and controls and serum was separated and stored at −70°C until cytokine assay. IFN-γ was chosen to represent Th1 pattern. According to Mosmann et al.,10 Ochoa et al.,11 O’Garra,12 like IL4, 5 and 10, IL6 is produced by Th2 cells. For reasons of availability and feasibility, IL6 was studied.

Both cytokines were measured by ELISA technique (Becton Dickinson, USA). The data was analysed and students’ unpaired t-test was used to compare patient groups with control group. The correlation coefficient (r) was applied between serum cytokine levels and different variables in the patient groups.

The study proforma was submitted to the Ethics Committee of B.J. Medical College, which is headed by the Dean and consists of representatives from the Departments of Medicine, Pharmacology, Gynaecology etc. as members. This committee holds meetings every 3 months and our study was considered at one such meeting, discussed and cleared thereafter. The funding for the cytokine kits was provided by the Departments of Dermatology and Microbiology.

Results and Observations

In the patient group, the mean serum levels of both cytokines (INF-γ and IL6) were significantly higher than the control group (except IL6 in BT and PN and INF-γ in LL) (Table 1). In the patient group, pure neuritic leprosy patients had lowest levels of IL6 and highest levels of IFN-γ followed by borderline tuberculoid group, while LL group had highest IL6 (Table1).

Comparing the two parts of the leprosy spectrum (Table 2), BT patients had significantly higher levels of INF-γ (P < 0.001) and significantly lower levels of IL6 (P < 0.001) as compared to LL patients.

Pure neuritic patient (PN) group had significantly higher levels of IFN-γ (P < 0.005) and significantly lower levels of IL6 (P < 0.005) as compared to BT patients. PN group had significantly higher levels of INF-γ (P < 0.001) and significantly lower levels of IL6 (P < 0.001) as compared to LL patients. LL group had significantly higher levels of IL6 as compared to controls (P < 0.001) and BT group (P < 0.001). A significant negative correlation was noted between the levels of INF-γ and IL6 (P < 0.001) in all the non reactional patients.

In the pure neuritic group, IL6 levels were inversely proportional (P < 0.005, r = −0.76) and IFN-γ levels were directly proportional (P < 0.001, r = +0.93) to number of nerves involved. (Table 3)

In lepromatous group (BB-BL-LL), bacteriological index was directly proportional to IL6 levels (P < 0.001, r = +0.82) and inversely proportional to INF-γ levels (P < 0.001, r = −0.58)

Type 1 reactional patients had higher levels of INF-γ and lower levels of IL6 as compared to other patients liable to such reactions (BT-BB-BL).

Only one patient of Type 2 reaction was included in our study. This patient had higher levels of both IL6 and IFN-γ as compared to other patients liable to such reactions (BL-LL). However as the number of patients in reaction was small, statistical significance of these observations could not be commented upon.
Table 1. Comparative mean serum cytokine levels in control group and different parts of the leprosy spectrum

<table>
<thead>
<tr>
<th>Pg/ml</th>
<th>Control</th>
<th>PN</th>
<th>BT</th>
<th>BB-BL</th>
<th>LL</th>
<th>BT with Type I</th>
<th>BL Type I</th>
<th>BL Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFγ</td>
<td>125.48 ± 26.28</td>
<td>1155.8 ± 149.35***</td>
<td>897.34 ± 2.57***</td>
<td>619.89 ± 152.51**</td>
<td>126.7 ± 31.32 NS</td>
<td>1130.3 ± 442.5</td>
<td>458.9</td>
<td>752.6</td>
</tr>
<tr>
<td>IL 6</td>
<td>20.5 ± 4.94</td>
<td>513 ± 2.57 NS</td>
<td>20.04 ± 4.52 NS</td>
<td>247.27 ± 148.92**</td>
<td>585.1 ± 38.2***</td>
<td>1673 ± 11.2</td>
<td>323.6</td>
<td>362</td>
</tr>
</tbody>
</table>

* ***P < 0.001, **P < 0.01, *P < 0.05, NS: not significant.
Leprosy is a disease which presents as a clinical and immunological spectrum between two poles. Most patients have the intermediate forms of BT, BB and BL leprosy which are associated with a progressive reduction from BT to BL in cellular responses, an increasing bacillary load, more skin and nerve lesions and higher antibody titres. The Th1 cells activated at the tuberculoid pole produce a strong CMI while Th2 cells activated at lepromatous pole inhibit Th1 cells resulting in defective CMI specific for *M. leprae*.

There are varied and conflicting reports regarding the status of Th1/Th2 subsets in leprosy patients. In the first report on skin lesions (Yamamura *et al.* 1992) mRNA coding for Th1 cytokines was evident in TT while Th2 cytokines were predominant in LL lesions indicating that resistance and susceptibility could be co-related with cytokine profiling. Subsequently, both polarised, mixed as well as varying combinations of Th1 and Th2 cytokines were reported in circulation and in lesions. Hence it is debatable whether a clear dichotomy exists between cytokine patterns and clinical forms of leprosy.

In our study, the mean levels of cytokines in patient group were significantly higher than control group (except INFγ in LL and IL6 in BT and PN which were not statistically different from controls), indicating immune stimulation by *M. leprae* in all parts of the spectrum. In comparison with other parts of the spectrum, non reactional pure neuritic patients had highest levels of INFγ and lowest levels of IL6 indicating that there could be maximum stimulation of CMI and activation of Th1 cells.

Even in the absence of clinical evidence of reaction, pure neuritic patients had INFγ levels equivalent to reactional BT patients. This could suggest that these patients are probably in a state of hypersensitivity with a possibility of greater nerve damage. Significantly, the levels of INFγ were found to be directly proportional to number of nerves involved. This could hint at a tuberculoid picture in our patients of pure neuritic leprosy having more extensive nerve involvement. A nerve biopsy and lepromin test would be required to know

### Table 2. Comparison of mean serum cytokines between BT and LL patients

<table>
<thead>
<tr>
<th></th>
<th>BT</th>
<th>LL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

***p < 0.001

### Table 3. Correlation between serum cytokine levels and nerve involvement in pure neuritic leprosy group

<table>
<thead>
<tr>
<th>No. of nerves involved</th>
<th>INFγ (pg/ml)</th>
<th>IL6 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single nerve (n = 3)</td>
<td>981.67 ± 19.21</td>
<td>6.2 ± 2.56</td>
</tr>
<tr>
<td>Two nerves (n = 4)</td>
<td>1147.75 ± 78.04</td>
<td>5.8 ± 3.91</td>
</tr>
<tr>
<td>Four nerves (n = 4)</td>
<td>1294.75 ± 43.27</td>
<td>3.6 ± 2.66</td>
</tr>
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n: number of patients

**Discussion**

Leprosy is a disease which presents as a clinical and immunological spectrum between two poles.

Most patients have the intermediate forms of BT, BB and BL leprosy which are associated with a progressive reduction from BT to BL in cellular responses, an increasing bacillary load, more skin and nerve lesions and higher antibody titres. The Th1 cells activated at the tuberculoid pole produce a strong CMI while Th2 cells activated at lepromatous pole inhibit Th1 cells resulting in defective CMI specific for *M. leprae*.

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the exact location of these patients in the spectrum. However, due to paucity of studies on
cytokines in pure neuritic patients, there are no reports available for comparison.

Various workers have attempted to classify pure neuritic leprosy on a clinical,
immunological and histopathological basis. Panniker et al. found that 41.2% and 58.8% of the
patients in their study belonged to lepromatous and non-lepromatous groups respectively. Later, Uplekar et al. observed that all their pure neuritic patients showed a narrower histological spectrum, ranging from TT-BB only. In contrast, Kaur et al. in a similar study demonstrated that none of the clinical parameters (including number and distribution of affected nerves), the immune response and the nerve histology were inter-related.

The higher levels of INFγ in BT compared to BB/BL/LL are compatible with the levels reported by Moubasher et al. in a similar study. Thus the main actions of INFγ are amplification of T cell response and marked alteration in behaviour of infected macrophages. This Th1 cytokine is known to cause activation of macrophages by bringing about biochemical, phenotypic and functional changes which translates into increased microbicidal activity. The protective value of INFγ was further demonstrated by the reduction of viable M.leprae at the site of intradermal injection of INFγ as shown independently by Kaplan et al. and Siva Sai et al.

On the other hand, IL6 levels were found to be highest in LL and lowest in PN group. Previous studies have shown that Th2 cells are activated in LL patients with production of IL 4,5,6,10 in skin lesions and peripheral mononuclear cells. Ochoa et al. have reported that 91% of LL patients produced IL6 in comparison to only 33% of tuberculous leprosy patients in their study. These Th2 cytokines are reported to inhibit Th1 cytokine production (particularly INFγ) and vice versa, which explains the significant negative co-relation between serum levels of INFγ and IL6 in all types of leprosy patients in our study. More studies on IL6 in leprosy are required to elucidate if like other Th2 cytokines (particularly IL10), IL6 is also an immunosuppressive molecule which inhibits macrophage mediated destruction of intracellular pathogens leading to increased bacillary load. So, if so, would substantiate the significant positive co-relation between IL6 and bacillary index noted in the BB-BL-LL group in our study. As quoted by Ochoa et al. and Seghal et al., IL 6 is known to promote antibody production and therefore the observed elevated levels of this cytokine may have some bearing on the hypergammaglobulinemia that characterises the lepromatous part of the spectrum.

Type I reactions or reversal reactions which occur in patients with borderline forms of disease are caused by spontaneous increases in T cell reactivity to mycobacterial antigens. They are associated with infiltration of INFγ and TNFα secreting CD4 lymphocytes in skin lesions and nerves resulting in edema and painful inflammation. In our study we encountered higher serum levels of INFγ in BT leprosy in Type I reaction as compared to non reactional patients. Moubasher et al. state that the elevated levels of INFγ in type I reaction indicate an exaggerated cell mediated immune response which culminates in clearing of bacilli and concomitant tissue damage. However, Faber et al. have reported a lack of demonstrable consistency in relation to cytokine levels and reversal reactions.

Type II reaction is a systemic inflammatory response to the deposition of immune complexes which occurs in BL and LL leprosy. The single type II reactional patient in our study had higher levels of IL6 compared to all other patients. IL6 is known to enhance B cell responses and augment antibody formation, thereby potentiating immune complex formation. Interestingly, this Type II reactional patient also had slightly elevated levels of INFγ as compared to nonreactional BL-LL patients. Moubasher et al. have reported a
similar finding in their Type II reaction patients, which points to a possible role of cell mediated immunity in the pathogenesis of Type II reaction. Moraes et al. have also admitted the possibility of transient INF gamma production by Th2 cells in tissue of ENL patients during Type II reactions. As the number of patients with reaction in our study was small, it is difficult to draw any definitive conclusions based on this data.

Conclusions

A current rapidly advancing area of mycobacterial research is the identification of correlates (in vivo or in vitro) of protective immunity to these organisms. Recent studies, such as by Lima et al. indicate that cytokine responses (most prominently the INF response) may provide useful markers of immune responses. Our study measured serum levels of cytokines in untreated leprosy patients and detected high levels of INF gamma in the tuberculoid part of the spectrum. Thus our findings are consistent with the hypothesis that INF gamma is immunoprotective in leprosy. The lepromatous leprosy patients had highest levels of IL-6.

In pure neuritic leprosy, elevated levels of INF gamma are associated with more extensive nerve involvement. To the best of our knowledge, this is probably one of the first studies of its kind in pure neuritic type which comprises a significant proportion of leprosy cases in the Indian scenario. Defining biological markers to predict the course of events in this group of patients remains a research priority and a goal to be achieved.

On this background, recombinant INF gamma, antibodies against IL-6 and drugs and vaccines acting on these cytokines could be potential immunochemotherapeutic tools in the perpetual battle against this morbid disease.

Therefore, future larger scale studies are warranted to further illuminate the role of cytokines in classification, prognosis and treatment of leprosy.

References


