Increased Serum Circulatory Levels of Interleukin 17F in Type 1 Reactions of Leprosy

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Abstract

Purpose Leprosy is a chronic infectious disease caused by Mycobacterium leprae affecting mainly skin and peripheral nerves. Acute inflammatory episodes in the borderline immunological spectrum of the disease cause severe nerve and tissue damage leading to deformities. Finding of any serological marker for leprosy reactions will help in prediction of reactions and in early treatment intervention. The objective of this study was to measure the serum circulatory levels of Interleukin 17F (IL 17F) and to correlate the levels with type 1 and type 2 reactional states and with clinico-histopathological spectrum of leprosy. We studied IL 17F to delineate its role and its clinical implications in leprosy reactions.

Methods Patients were classified based on the Ridley DS and Jopling WH Classification and blood samples (5 ml each) were collected from 80 active untreated leprosy cases in Type 1 reaction (T1R), 21 cases in Type 2 (Erythema Nodosum Leprosum ENL) reaction (T2R), 80 cases without reaction (NR), and 94 non-leprosy cases (NL). Serum was separated and measured for IL 17F levels using ELISA (Commercial Kits, R&D Systems Inc., USA).

Results IL 17F levels were significantly higher in the T1R group when compared to the NR group \( (p < 0.001) \). The borderline lepromatous group showed the highest levels of IL 17F among the other groups in the disease spectrum. Bacteriological index (BI) showed negative correlation with the IL 17F levels.

Conclusion The results specify that serum circulatory levels of IL 17F are elevated during T1Rs in the borderline spectrum of the disease and thus may play a role in the regulation of inflammatory responses associated with reactions in leprosy.

Keywords Proinflammatory cytokines · type 1 reactions · Ridley DS and Jopling WH Classification · delayed type hypersensitivity reactions · bacteriological index (BI) · Erythema Nodosum Leprosum (ENL)

Introduction

Leprosy is a chronic infectious disease caused by an intracellular obligatory organism, Mycobacterium leprae that affects mainly the peripheral nerves, skin, and mucous membranes and has inflicted human beings from the biblical times in most parts of the world. It is now limited only to tropical and subtropical countries [1]. Although the worldwide prevalence of leprosy has been brought down to <1 per 10,000 population size during the era of multidrug therapy (MDT), the new cases are occurring almost at the same rate in many situations, especially in India and in Brazil indicating the existence of active man to man transmission [2]. Approximately 0.126 million new leprosy cases have been detected in India in 2010 out of which 3,927 cases are with visible (grade 2) disabilities [3]. In our Leprosy Mission referral hospitals, more than 25 % of patients have been reported with visible (grade 2) disabilities and another 20% with anesthesia and/or a high bacteriological index (TLM Annual Report 2010).

It has been established beyond doubt that host immunity determines the outcome of clinical manifestations in leprosy. At the tuberculoid (TT) pole strong resistance exhibited by the
host cell mediated immunity (CMI) limits the growth of M. leprae in a few granulomatous areas in the skin and nerve with limited multiplication of the bacilli while at the lepromatous (LL) pole the host M. leprae specific CMI barriers are tresspassed by the overgrowth of bacteria leading to the breakdown of the defense of the host [4]. As the human host immune system is always in a dynamic phase of variation, the disease also gets manifested according to the type of the immune status of the host in various borderline clinical forms called borderline tuberculoid (BT); borderline-borderline (BB) and borderline lepromatous (BL) between the two poles, Tuberculoid (TT) and Lepromatous (LL). This classification was further standardized on a clinico-histopathological, bacteriological, and immunological scale (Ridley DS and Jopling WH, 1960) [5]. Pure neuritic leprosy (PN) was characterized by neural impairment (neuropathy) without evidence or history of typical skin lesions. However, during these borderline spectral clinical manifestations, acute inflammatory episodes may also occur during and after MDT, which are known as “reactions in leprosy” [6].

The T1R is characterized by an increase in inflammation in the skin and nerves. This type of reaction predominantly occurs in the borderline states of leprosy. A small number of leprosy cases towards the polar forms also experience these reactional states. Skin lesions become erythematous and/or edematous and may ulcerate. Edema of the hands, feet, and face can also be a feature of a reaction but systemic symptoms are unusual. Acute neuritis may develop and it leads to nerve function impairment (NFI) which when not treated rapidly and adequately will lead to permanent loss of nerve function [7].

A T2R or ENL on the other hand usually occurs in the borderline lepromatous and lepromatous groups with cutaneous manifestations of widespread or deep crops of inflamed erythematous nodules and papules. Further, the occurrence of ulcerated, necrotic, pustular, and bullous forms has also been reported. Some nodules may persist as a chronic painful panniculitis leading to fibrosis and scarring [13].

IL-17F is an important member of the group of cytokines called Interleukin-17 family. It acts as a potent mediator in the delayed—type hypersensitivity reactions to recruit monocytes and neutrophils to the site of inflammation by increasing chemokine production in various tissues which is similar in function to Interferon gamma [8]. It is produced by T helper cells and is induced by IL-23 which results in destructive tissue damage in delayed—type reactions [9]. Interleukin 17 as a family functions as a proinflammatory cytokine that responds to the invasion of the immune system by extracellular pathogens and induces destruction of the pathogen’s cellular matrix [10]. IL-17 is an active recruiter of neutrophils to inflammatory sites and IFN gamma regulates the induction of Th17 cells. This may explain the damaging inflammatory response seen during mycobacterial infection of IFN gamma deficient mice indicating that IFN-gamma and IL-17F may counter-regulate each other during chronic mycobacterial infections [11].

Owing to the capabilities of IL 17F to potentially induce chemokine expression and recruit cells to parenchymal tissues and the involvement of Th1 and Th17 responses which cross regulate during mycobacterial infections [12], we have chosen to study the serum circulating levels of IL 17F in leprosy cases with reactions and compare them with non-reaction leprosy cases (control group) and also correlate them with the clinico-histopathological classification and with the bacteriological index in leprosy.

**Methods**

**Patients and Controls**

The study population comprised of active untreated leprosy cases in T1R (n=80), cases in T2R (n=21), NR cases (n=80) (control group) and NL cases (n=94) (recruited for estimation of normal values and threshold data). The demographic and clinical characteristics of the cases and the controls were shown in (Table 1).

Leprosy cases were randomly selected from the patients attending the outpatient department of The Leprosy Mission Community Hospital—Shahdara, New Delhi. The ethics committee of The Leprosy Mission Trust India approved the study protocol. After the participants signed informed consent, they underwent thorough clinical assessment for dermatological and medical complications. Slit skin smears were collected and acid fast staining was performed to determine the bacteriological index (BI). Following clinical and histopathological examination, the cases were divided based on the Ridley DS and Jopling WH classification into TT—(Tuberculoid), BT—(Borderline Tuberculoid), BL—(Borderline Lepromatous), LL—(Lepromatous) and PN—(Pure Neuritic) groups. Based on the BI, the cases were divided into negative BI, BI between 0.1–3 and 3.1–6 groups.

Common clinical manifestations of cases with T1R and T2R include the presence or sudden appearance of erythematous patches that are tender and swollen accompanied by malaise and edema of hands and feet. Symptomatically, cases with T1R manifested acute inflammation in the skin and nerves and cases with T2R manifested wide spread crops of erythematous nodules and papules which are inflamed and are either present superficial or deep.

All cases on multi drug therapy and/or on steroids are excluded from the study. Non-leprosy cases include healthy volunteers and cases with medical conditions other than dermatological and inflammatory complications. 5 ml of peripheral venous blood in EDTA was collected from each subject using sterile syringe and serum was separated and stored in −70 °C until IL 17F ELISA was performed.
ELISA for IL 17F

Enzyme Linked Immunosorbent Assay (ELISA) for IL 17F was carried out using commercial kits from R&D Systems Inc., Minneapolis, MN, USA (Cat No: DY1335) as per the manufacturer’s instructions. Briefly, 96 well microtitre plates were coated with working concentration of 0.8 μg/ml of capture antibody (goat anti-human IL 17F) diluted in phosphate buffer saline (PBS) without carrier protein. The plates were incubated at room temperature for overnight and washed with wash buffer containing 0.05 % Tween 20 in PBS, pH 7.2 – 7.4 (R&D Systems Catalog # WA126). Washing includes aspiration of each well followed by filling with wash buffer up to 400 μl per well using an auto washer. This process was repeated 3 times and complete removal of the liquid was ensured at each step by inverting the plates and blotting them on a filter paper towel. The plates were then blocked for 1 h with 300 μl of reagent diluent containing 1 % BSA in PBS, (pH 7.2–7.4, 0.2 μm filtered) (R&D Systems Catalog # DY995). After washing, samples and standards with dilution of samples as 1: 2 and a 7-fold dilution of standards with the highest concentration being 20,000 pg/ml were added to the appropriate wells (diluted in reagent diluent). Samples were loaded in duplicates and the plates were incubated for 2 h at room temperature. This was followed by washing and addition of 100 μl/well of detection antibody (biotinylated goat anti-human IL-17F) at a working concentration of 100 ng/ml diluted in reagent diluent. After incubation for 2 h at room temperature, the plates were washed and 100 μl/well of Streptavidin-Horse Radish Peroxidase conjugate (working dilution of 1:200 in reagent diluent) was added and incubated for 20 min in the dark. Plates were washed again and 100 μl/well of substrate solution containing 1:1 mixture of Color Reagent A (H2O2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems Catalog # DY999) was added and incubated for 20 min in the dark. Then the reaction was stopped by adding 2N H2SO4 and the optical density was determined using Dynatec ELISA plate reader at a wavelength of 450 nm with a reference filter at 570 nm.

Statistical Analysis

Mean serum levels of IL 17F in pg/ml were measured in all the groups mentioned above and the results were analyzed by pairwise and multiple comparisons using Kruskal Wallis Non-Parametric Test for multiple comparisons. The pairwise comparisons between the groups were done with Fischer’s Least Significant Difference contrast.

Results

IL 17F Levels in Leprosy Reactions

The mean serum levels of IL 17F were significantly high in the T1R group \((p<0.05)\) when compared to NR and NL groups. There was also a significant upsurge in the IL 17F levels in the NR group when compared to the NL and in the T2R group when compared to the NL group. However, no significant difference was observed in IL 17F levels between the T1R and T2R \((p=0.06)\) and between the T2R and the NR groups \((p=0.37)\) (Table II).
IL 17F Levels in Different Classes of Ridley DS and Jopling WH Classification

Association analysis of IL 17F levels with immunological spectrum of leprosy revealed that the BL group showed significantly high levels when compared to TT, BT and LL groups \( (p<0.01, p<0.01 \) and \( p<0.009 \) respectively). However, there was no statistically significant difference observed in the IL 17F levels between the BL and PN, TT and BT, TT and LL, BT and LL and BT and PN groups \( (p>0.05) \).

Samples were also classified based on the bacteriological index (BI) into three groups, BI—Negative, BI 0.1–3 and BI 3.1–6. Pairwise comparisons between the three groups revealed that BI 0.1–3 group has significantly high IL 17F levels when compared to BI Negative \( (p=0.01) \) and BI 3.1–6 \( (p=0.0041) \) groups. However, there was no significant difference in levels between the BI Negative and BI 3.1–6 groups \( (p=0.16) \) (Table III).

Correlation of IL 17F Levels with BI

Correlation of IL 17F levels with BI 0.1–3 and BI 3–6 groups revealed that levels are inversely proportional to the BI and there was a significant negative correlation \( (r=-0.40) \) \( (p<0.001) \) (Fig. 1).

Discussion

Leprosy presents a spectrum of immunological groups between the two poles, the tuberculoid pole (TT) with increased cell mediated immunity which gradients towards the lepromatous pole (LL) though a series of borderline forms (BT, BB and BL) with simultaneous increase in humoral immune response and increase in the bacillary load.

T1Rs are delayed type hypersensitivity reactions, which occur predominantly in the borderline forms of leprosy. M. leprae antigens have been demonstrated in the nerves and skin of patients experiencing T1Rs [14, 15]. The antigens are localized in the macrophages and Schwann cells [16]. A study of Brazilian patients with slit-skin smear negative—single lesion paucibacillary leprosy showed that individuals with M. leprae DNA detectable by PCR in the skin were more likely to experience a T1R than those in whom M. leprae DNA was undetectable [17]. Schwann cells also express toll-like receptor (TLR) 2 [18]. M. leprae infection may lead to the expression of MHC II molecules on the surface of the cells and thus may participate in antigen presentation which may trigger CD4 lymphocyte mediated killing of the cell in the presence of cytokines such as IL 1β and TNF α [19].

The IL-17-producing T helper (Th17) cells have been recently identified as a new subset of the T helper cells

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<tr>
<th>Table II</th>
<th>Levels of IL 17F within the study groups T1R, T2R, NR and NL</th>
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<tr>
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<td>2. T2R</td>
<td>21</td>
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<tr>
<td>3. NR</td>
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<td>4. NL</td>
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*\( p<0.05 \) is considered as statistically significant

<table>
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<tr>
<th>Table III</th>
<th>Levels of IL 17F within the Ridley DS and Jopling WH Classification and Bacteriological Index</th>
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<td>Variables</td>
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<td>BL</td>
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<td>BI 0.1–3</td>
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<tr>
<td>BI 3.1–6</td>
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and as potential mediators of inflammation associated with various autoimmune diseases [20]. Although several cytokines participate in Th17 cell development, IL-6 and TGF-β are key factors for the generation of Th17 cells from naïve T cells [21]. IL-17F is expressed in Th17 cells and other types of IL-17 expressing T cells in-vivo. Several studies describe similar regulation of IL-17 and IL-17F expression by cytokines IL-23, TGF-β, IL-6, and IL-21, as well as transcription factors RORγt, RORα, and STAT3. IL 17F induces delayed type hypersensitivity reactions and it is produced not only by Th17/ThIL-17 cells, but also by activated CD8+ T cells, TCRγδ+ T cells, and neutrophils [22–26].

We have identified a statistically significantly high levels of IL 17F in the borderline forms of the disease BT, BL and T1Rs where there is a spontaneous increase in the Th1 response [27]. Recent studies revealed that IL 17F is not critical for the overall protection against the infecting pathogen but mediate inflammatory responses against some intracellular pathogens [28]. IL 17F mediating the Th1 response has been demonstrated in the mouse model, after infection with M. bovis BCG. The generation of IFN γ producing Th1 cells was reduced in the absence of IL 17 and neutrophil recruitment to the lung was impaired [11]. The mechanism by which IL-17 regulates the Th1 pathway appears to be via induction of IL-12 and IFN γ in antigen presenting cells [29]. Following IL-17 stimulation, both dendritic cells and macrophages were noted to liberate IL-12 and IFN γ leading to regulation of downstream immune responses [29].

Our results also indicated that the mean cytokine levels in all the groups of leprosy are significantly high when compared to the non-leprosy group. This may suggest that M. leprae stimulate the production of IL 17F across the spectrum of the disease. Patients having BI 0.1–3 showed higher levels of IL17F when compared to patients with negative BI indicating that the presence of optimum levels of M. leprae may activate the Th17cells to produce IL 17F. However, with the increase in the BI from 3 to 6, there was a fall in the IL 17F levels resulting in the significant negative correlation between the IL17F levels and the BI. It has been shown earlier that IL 17F inhibits the Th 2 response by inhibiting the production of IL5 and IL 13 and vice versa [30]. In the present situation, whether the increase in bacillary load from 3 to 6 leads to a shift in Th1 to Th2 response with lowering the levels of IL17F, is beyond the scope of this study and has to be explored. Recent studies [31] revealed that Th 17 cells were involved in the immunopathogenesis of ENL reactions and IL 17F gene expression was up regulated before and after thalidomide treatment (a drug administered for the resolution of ENL reactions). Our studies are concurrent with these findings owing to the significant upsurge in IL 17F levels in cases with ENL reaction (type 2 reaction) when compared with the non-leprosy cases.

Further functional analysis needs to be pursued in the context of understanding the IL 17 mediated immunomodulatory mechanisms involved in regulation of inflammation and such a study may provide deeper insights into the functional role of IL 17F in the control of type 1 reactions in leprosy.

A prospective cohort study involving measurement of IL 17F levels, before, during and after the occurrence of type 1 reactions and during MDT treatment may provide predictive information on the effectiveness of IL 17F to identify patients going to manifest type 1 reactions in Leprosy.

**Conclusion**

One of the rapidly advancing areas of mycobacterial research include identification of early disease detection tools such as predictive and prognostic biomarkers which may help in early identification and treatment of nerve and tissue damage associated with this morbid disease—Leprosy. Circulating profiles of the cytokines may act as potential plasma markers [32] to identify the disease early and predict the occurrence of reactions. Various research groups [33, 34] in this context have studied key proinflammatory and inflammatory cytokines but one has to explore the novel T cell subsets and their cytokines. In this study, we propose a possible association of one the recently identified cytokine—IL 17F of the interleukin 17 family of cytokines which are reported to have a potential role in the immunomodulation of inflammatory responses [35]. Our results are concurrent with our hypothesis that IL 17F levels increase during reactional states in leprosy when compared with the non-reactional cases. Presence of optimum levels of M. leprae may elicit the production of this proinflammatory cytokine whose functional role is yet to be deciphered in the context of understanding the IL 17 mediated immunomodulatory mechanisms involved in regulation of inflammation.
of leprosy for further association with the disease prognosis and classification.

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Declaration of Interest The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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