CIRCULATORY AND LOCALIZED mRNA EXPRESSION PROFILES OF INTERLEUKIN 17F AND INTERLEUKIN 23 IN TYPE 1 (REVERSAL) REACTIONS OF LEPROSY

1 - Stanley Browne Laboratory, The Leprosy Mission Trust India, New Delhi, India
2 - The Leprosy Mission Community Hospital, The Leprosy Mission Trust India, Nand Nagri, New Delhi, India - India

*Sundeep Chaitanya
Mallika Lavania
Ravindra Turankar
Asthana Nigam
Iltu Singh
Ilse Horo & Utpal Sengupta

*Presenting Author
• Borderline forms of leprosy is often characterized by immune exacerbations called Type 1 (Reversal) reactions which occurs in 30-40% of cases at the time of diagnosis or during MDT.

• Interleukin 17F (IL 17F) and Interleukin 23 (IL 23) are the proinflammatory cytokines that play a crucial role in the regulation of peripheral inflammations and the pathway is critical for the development of auto-immune inflammatory diseases.

• In this study, we investigated the possible association of serum circulatory levels, T cell responses, lesional skin mRNA expression levels of IL 17F and IL 23 and functional single nucleotide polymorphism of IL 17F (+7488 T/C) with manifestation of type 1 Reactions in Leprosy.
IL 17/IL 23 AXIS IN INFLAMMATION

IL-12p70

p35

p40

α-p40

T_{H1} cell

IFN-γ

Host immunity to bacterial and viral infections and tumor surveillance

T_{H17} or γδ T cell

IL-17A

Autoimmunity to fungal and extracellular bacterial infections

IL-23

p19

α-p19

p40

IL-1, IL-6

α-IL-17A

α-IL-17RA

α-IL-17F
OBJECTIVES

- To investigate the functional role of Interleukin 17 and Interleukin 23 in the regulation of peripheral inflammations in leprosy by studying:
  
  - The serum circulatory profiles of IL 17F and IL 23 in Type 1 Reactions (T1R) of leprosy.
  - The secretory profiles of IL 17F and IL 23 in mononuclear cell cultures stimulated with *M. leprae* Soluble Antigen (MLSA).
  - mRNA expression profiles of IL 17F and IL 23 in reactional skin lesions reflecting their localized activity in peripheral inflammations.
  - Association of +7488 T/C Functional Single Nucleotide Polymorphism (SNP) in IL 17F gene with manifestation of Type 1 Reactions in Leprosy.
**METHODOLOGY**

Newly Diagnosed Untreated Leprosy Cases (Both T1R and NR)

- Informed Consent for Participation as per ICMR Guidelines

- Serum Levels of IL 17F and IL 23 (T1R n=69, NR n=76)
  - ELISA for Serum levels of IL 17F and IL 23

- T Cell & Whole Blood Assays with MLSA (T1R n=24, NR n=30)
  - ELISA For IL 17F and IL 23 in Cell Culture Supernatants
  - Genotyping +7488 T/C SNP in IL 17F gene

- 5mm X 5mm Lesional Skin Biopsy samples (T1R n=27, NR n=29)
  - TRI Reagent Protocol
  - Total RNA extraction & cDNA Construction
  - Realtime PCR based quantification of mRNA of IL 17F and IL 23

*T1R = Leprosy Cases in Type 1 Reaction
*NR = Leprosy cases not in Reaction
• Serum levels of IL 17F (T1R Vs NR, 202.0 pg/ml Vs 165.5 pg/ml, p<0.05).
• Serum levels of IL 23 (T1R Vs NR, 64.57 pg/ml Vs 75.54 pg/ml, p>0.05). (Mann Whitney U Test)
IL 17F AND IL 23 LEVELS ACROSS STUDY GROUPS (T1R & NR) (IN-VITRO WHOLE BLOOD ASSAYS WITH MLSA*)

- Cell culture supernatant (CCS) levels of IL 17F (T1R Vs NR, 530.7 pg/ml Vs 310.1 pg/ml, p<0.05).
- CCS levels of IL 23 (T1R Vs NR, 297.4 Vs 281.8, p>0.05). (Mann Whitney U Test)

*M.leprae soluble antigen
IL 17F AND IL 23 mRNA EXPRESSION PROFILES IN LESIONAL SKIN BIOPSY SAMPLES

- Realtime Quantification of IL 17F and IL 23 mRNA expression was done using relative quantification - Pfaffl method.
- Primer efficacies were determined for GAPDH as Reference gene and for IL 17F and IL 23. (GAPDH = 98%, IL 17F = 97%, IL 23 = 91%)
- 1 μg of total RNA extracted from the biopsy samples was converted to cDNA using commercial Kits (New England Biolabs). Standard Graphs were constructed with 6 fold dilutions of cDNA to determine the primer efficacies.
- The fold difference was identified using the formula:

\[
\text{Ratio} = \frac{(E_{\text{target}})^{\Delta C_{\text{target}}}}{(E_{\text{ref}})^{\Delta C_{\text{ref}}}} \frac{\text{Control} - \text{Sample}}{\text{Control} - \text{Sample}}
\]
mRNA expression ratios of IL (T1R Vs NR, 7.17 Vs 1.43, p<0.05).
mRNA expression ratios of IL 23. (T1R Vs NR, 2.76 Vs 2.06, p>0.05). *(Mann Whitney U Test)*
ANALYSIS OF FUNCTIONAL SNP (+7488 T/C) IN INTERLEUKIN 17F GENE AND ITS ASSOCIATION WITH MANIFESTATION OF TYPE 1 REACTIONS IN LEPROSY

<table>
<thead>
<tr>
<th>Group</th>
<th>T/T Genotype</th>
<th>T/C Genotype</th>
<th>C/C Genotype</th>
<th>T Allele</th>
<th>C Allele</th>
<th>p-Value (1+2 Vs. 3)</th>
<th>OR (95% CI) (1+2 Vs. 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Leprosy PB (n=53)</td>
<td>42 (79.24%)</td>
<td>9 (16.98%)</td>
<td>2 (3.77%)</td>
<td>88%</td>
<td>12%</td>
<td>(T/T) &lt;0.0001</td>
<td>(T -Allele) 6 (CI:2.91 – 12.3)</td>
</tr>
<tr>
<td>(2) Leprosy MB (n=87)</td>
<td>68 (78.16%)</td>
<td>16 (18.39%)</td>
<td>3 (3.44%)</td>
<td>87%</td>
<td>13%</td>
<td>(T/C) &lt;0.0001</td>
<td>(C - Allele) 0.16 (CI:0.08 - 0.34)</td>
</tr>
<tr>
<td>(3) Controls (n=120)</td>
<td>25 (20.83%)</td>
<td>84 (70.00%)</td>
<td>11 (9.17%)</td>
<td>55%</td>
<td>45%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All the leprosy cases in TT (n=110) and TC (n=25) genotypes were followed over a period of 12-24 months for the development of T1R during the course of MDT and/or steroid treatment.
ASSOCIATION OF IL 17F (+7488 T/C) GENOTYPES WITH MANIFESTATION OF TYPE 1 REACTIONS IN LEPROSY

Out of the 110 cases with TT genotype, 82 (74.54%) cases developed T1R whereas out of 25 cases with TC Genotypes, 8 (32.00%) cases developed T1R (74.54% vs 32.00%, \( p<0.05 \)).
SALIENT OBSERVATIONS

• We observed statistically significant higher serum and CCS levels of IL 17F in leprosy cases with Type 1 Reaction (T1R) when compared to cases not in Reaction (NR).

• We observed statistically significant higher mRNA expression ratios of IL 17F in lesional skin specimens from leprosy cases with T1R.

• No relative differences in the circulatory, secretory and gene expression profiles of IL 23 was observed across the study groups.

• Significantly higher number of leprosy cases with TT genotype for IL 17F (+7488 T/C) SNP developed T1R when compared to those with TC genotype.
CONCLUSION

• Our study indicated an association of systemic and localized levels of IL 17F with the development of T1R in leprosy.

• Differential mRNA expression profiles of IL 17F and IL 23 provide insights into their role in regulating peripheral skin reactions in autoimmune inflammations as that of T1R in leprosy.

• Our observations on in-vitro liberation (Whole Blood Assays) of IL 17F/IL 23 and association of (+7488 T/C) SNP of IL 17F gene with T1Rs may aid in developing T cell based and genetic markers for laboratory based early diagnosis of T1Rs in leprosy.

FUTURE RECOMMENDATIONS

• Functional analysis of Interleukin 17F in a longitudinal study design may aid in identifying its potential role as serological, in-vitro T cell based, gene expression and genetic (SNP) marker for early diagnosis of T1Rs in leprosy there by preventing nerve damage and consequent deformities.
REFERENCES


ACKNOWLEDGEMENTS

Our Special thanks to The Leprosy Mission Trust – the host organization for its support throughout the study and the Indian Council of Medical Research for the financial support – IRIS ID No: 2010-12950