Role of Th17 cells in leprosy spectrum

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**Materials and Methods**

### Subjects

<table>
<thead>
<tr>
<th>Clinical groups</th>
<th>Age in years</th>
<th>Sex (M/F)</th>
<th>Duration of disease (months)</th>
<th>BI</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT (19)</td>
<td>19-59</td>
<td>13/6</td>
<td>1-36</td>
<td>0-1+</td>
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<tr>
<td>LL (18)</td>
<td>24-60</td>
<td>13/5</td>
<td>6-12</td>
<td>5-6+</td>
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<td>HC (5)</td>
<td>22-40</td>
<td>3/2</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Normal skin (4)</td>
<td>22-28</td>
<td>4/ -</td>
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### Methods

1. qPCR for expression of Th17 associated genes /markers
   - *In situ* dermal lesions
   - Stimulated 48hrs PBMC cultures with armadillo derived MLSA

2. Phenotype of Th17 cells by flow cytometry

3. ELISA for cytokines
Heat Map of gene expression in PBMC and skin lesions

Time Kinetics
48h cultures

In situ
Increase in IL17, RORC expression in tuberculoid leprosy and healthy contacts - qPCR

A

B

RORC

PBMC

SKIN

p<0.0007

p<0.0002

p<0.001

p<0.004

p<0.003

p<0.0007

p<0.002

p<0.0001

p<0.0007

p<0.002

p<0.0001

p<0.0007

p<0.01

p<0.02
Expression of IL-21, 22, 23 support Th17 - qPCR

- IL-21
  - PBMC
  - SKIN
  - $p < 0.001$
  - $p < 0.0002$
  - $p < 0.0007$

- IL-22
  - PBMC
  - SKIN
  - $p < 0.002$
  - $p < 0.0007$

- IL-23A
  - PBMC
  - SKIN
  - $p < 0.001$
  - $p < 0.0003$
  - $p < 0.007$

- IL-23R
  - PBMC
  - SKIN
  - $p < 0.001$
  - $p < 0.0007$

The ΔCt values for IL-21, 22, 23, and IL-23A and IL-23R are shown for PBMC and SKIN samples, with statistical significance indicated by $p$-values.
What is Th17 cell?
1. IL17+ cells belong to CD4+ subset of CD3+ T cells
2. IL17A+ cells increased in BT leprosy
3. IL21+ CD4+ T cells increased in BT leprosy
Is Th17 an effector/memory Cell?

1. IL 17+ cells belong to CCR6+ effector T cell lineage

2. CCR6+ IL17+ cells increased in BT leprosy
1. Total STAT3 similar expression.
2. Phosphorylation of STAT 3 is a prerequisite for IL17 production.
ELISA: Cytokine levels in culture supernatants of stimulated PBMC support Th17 cells in BT patients

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<th>Cytokines</th>
<th>BT (10)</th>
<th>LL (11)</th>
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<tr>
<td><strong>Mean pg/ml ± SD</strong></td>
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<tr>
<td>IL-17A/F</td>
<td>101.9±26.28***</td>
<td>45.5±22.07</td>
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<td>IL-21</td>
<td>314.2± 19.18***</td>
<td>267.4± 11.5</td>
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<td>IL-22</td>
<td>561.4± 118</td>
<td>633.1± 89.18</td>
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<tr>
<td>IL-23</td>
<td>62.65± 51.25**</td>
<td>22.5± 11.4</td>
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<td>IL-6</td>
<td>50.5±27.9***</td>
<td>21.8±10.9</td>
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<td>IL-1β</td>
<td>13.6±16.34</td>
<td>8.58±4.4</td>
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<tr>
<td>IFN-γ</td>
<td>749.7±1314**</td>
<td>53.12±23.3</td>
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<td>IL-5</td>
<td>9.24± 9.39</td>
<td>36.4± 14.46***</td>
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<td>IL4 (ΔCt)</td>
<td>5.4</td>
<td>4.4</td>
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<td>IFNγ (ΔCt)</td>
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**What is the relationship of Th17 to polarised Th1 & Th2, phenotypes?**

**Th17 shows correlation with non polarized Th0 phenotype**
CONCLUSION

1. Th17 cells associate more with Th0 subset in leprosy NOT with polarised Th1 and Th2.

2. Form a third subset in leprosy

3. Role in control of disease prior to /or in absence of polarised Th phenotypes

THANK YOU

CD4+Th17 cells Discriminate Clinical Types and constitute a third subset of non Th1, non Th2 T cells in Human Leprosy

PLoS Negl Trop Dis 7(7): e2338. doi:10.1371/journal.pntd.0002338
1. CD8+ T cell subset produce negligible to low IL-17, IL-21 in leprosy
2. CD4+ T cells with IL-21 increased in BT leprosy