Global Gene Expression Studies of PBMC During Reversal Reactions Show Increased Expression of Microbial Recognition Receptors

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Research Objectives

1. To better understand the pathogenesis of reversal reactions of leprosy using global gene expression of peripheral blood mononuclear cells (PBMC) as a tool.

2. To develop model for autoimmune disease and immunity-related complications of other infectious diseases.
Host Gene Expression: Tuberculoid vs Lepromatous Skin Lesions

Tuberculoid
- Antigen Presentation
- Antimicrobial
- Th1

Lepromatous
- Anti-inflammatory
- Inhibitory receptors
- B cell response

Nature 2003;301:1527-30
Goals

1. Compare gene expression in peripheral blood mononuclear cells (PBMC) of borderline leprosy patients with or without RR using microarray.

2. Confirm differential gene expression in PBMC using qPCR of a validation set of borderline leprosy patients with or without RR.
New Case Detection in Rio Grande do Norte, Brazil

Map courtesy of Dr. Mauricio Nobre
Criteria for Inclusion in Microarray

Inclusion criteria
  – Confirmed diagnosis of leprosy
  – Confirmed clinical diagnosis of reversal reaction

Exclusion criteria
  – Less than 18 years of age
  – Systemic corticosteroid in prior 7 days
Methods

1. Isolation of PBMC from peripheral blood
2. RNA extraction and purification
3. **Phase 1**: Microarray analysis using Illumina HT12v4 chips
4. **Phase 2**: qPCR using thin-liquid density array (TLDA)
## Study Population: Microarray

<table>
<thead>
<tr>
<th></th>
<th>Reversal Reaction (RR, n=11)</th>
<th>Without Reversal Reaction (Non-RR, n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td>54.5%</td>
<td>54.5%</td>
</tr>
<tr>
<td><strong>Mean age in years (range)</strong></td>
<td>47.6 (22-73)</td>
<td>47.7 (28-65)</td>
</tr>
<tr>
<td><strong>Leprosy Treatment Status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>MDT</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Post-MDT</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Ridley-Jopling Classification</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>BB</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>BL</td>
<td>5</td>
<td>4</td>
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</tbody>
</table>
### Study Population: qPCR

<table>
<thead>
<tr>
<th></th>
<th>Reversal Reaction (RR, n=22)</th>
<th>Without Reversal Reaction (Non-RR, n=18)</th>
<th>RR vs. non-RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>59.1%</td>
<td>55.6%</td>
<td>p=0.892</td>
</tr>
<tr>
<td>Mean age in years (range)</td>
<td>50.1 (22-78)</td>
<td>50.7 (28-82)</td>
<td>p=0.822</td>
</tr>
<tr>
<td>Ridley-Jopling Class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT</td>
<td>3</td>
<td>2</td>
<td>p=0.960</td>
</tr>
<tr>
<td>BB</td>
<td>9</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>BL</td>
<td>10</td>
<td>8</td>
<td>-</td>
</tr>
</tbody>
</table>
Differential Gene Expression in RR vs. non-RR

There were 180 transcripts with FC ≥ 1.5 and p-value ≤ 0.05.

* Borderline leprosy with RR
+ Borderline leprosy without RR
Top KEGG Pathways in PBMC During RR

1. *Staphylococcus aureus* infection ($p_{adj} = 4.95 \times 10^{-5}$)
2. Systemic lupus erythematosus ($p_{adj} = 0.0021$)
3. Hematopoietic cell lineage ($p_{adj} = 0.0030$)
4. FcγR-mediated phagocytosis ($p_{adj} = 0.0034$)
# Complement in RR

<table>
<thead>
<tr>
<th>Gene</th>
<th>RR vs. control</th>
<th>Fold-change</th>
<th>p-value</th>
<th>Fold-change</th>
<th>p_adj</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1Q</td>
<td>RR up</td>
<td>1.57</td>
<td>0.065</td>
<td>1.72</td>
<td>0.040</td>
</tr>
<tr>
<td>C2</td>
<td>RR up</td>
<td>2.17</td>
<td>0.018</td>
<td>1.99</td>
<td>0.060</td>
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<tr>
<td>Serpin1</td>
<td>RR up</td>
<td>2.01</td>
<td>0.015</td>
<td>2.25</td>
<td>0.013</td>
</tr>
<tr>
<td>C5L2</td>
<td>RR up</td>
<td>1.60</td>
<td>0.022</td>
<td>1.43</td>
<td>0.024</td>
</tr>
</tbody>
</table>

**Microarray**

**TLDA validation**
# Monocyte Receptors in RR

<table>
<thead>
<tr>
<th>Gene</th>
<th>RR vs. control</th>
<th>Fold-change</th>
<th>p-value</th>
<th>Fold-change</th>
<th>$p_{adj}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcγRI</td>
<td>RR up</td>
<td>2.58</td>
<td>0.005</td>
<td>2.29</td>
<td>0.008</td>
</tr>
<tr>
<td>FPR1</td>
<td>RR up</td>
<td>1.70</td>
<td>0.017</td>
<td>1.86</td>
<td>0.007</td>
</tr>
<tr>
<td>FPR2</td>
<td>RR up</td>
<td>2.01</td>
<td>0.014</td>
<td>2.12</td>
<td>0.003</td>
</tr>
<tr>
<td>MARCO</td>
<td>RR up</td>
<td>1.84</td>
<td>0.036</td>
<td>1.97</td>
<td>0.017</td>
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</tbody>
</table>
Monocytes in MB Patients With or Without RR

- Graph showing the percentage of monocytes in PBMCs for RR and No Reaction groups.
- The graph indicates a higher percentage of monocytes in the RR group compared to the No Reaction group.
C1q in RR Skin Biopsies

BT skin biopsy

RR skin biopsy
New Hypotheses

1. Both innate and adaptive immune responses are important for reversal reactions.

2. Antigen, antibody, and antigen-antibody complexes may have roles in the immune response during reversal reactions.

3. More than one type of antigen or a response not targeted to a specific antigen may be involved in RR.
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