DNA Microarray Analysis of Human Monocytes Early Response Genes upon Infection with \textit{Rickettsia rickettsii}

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**Title:** DNA Microarray Analysis of Human Monocytes Early Response Genes upon Infection with Rickettsia rickettsii

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**Abstract:** See also ADM001849, 2004 Scientific Conference on Chemical and Biological Defense Research. Held in Hunt Valley, Maryland on 15-17 November 2004. The original document contains color images.
Rickettsiae

- Gram negative coccobacillary bacteria
- Obligate intracellular organisms
- Arthropod-borne
- Cause febrile diseases (mild to life threatening)
- Military importance
  - Epidemic Typhus: Peloponnesian war, Napoleon, WWI, WWII
  - Trench fever: WWI and WWII
  - Scrub typhus: WWII, Vietnam, Camp Fuji
  - Ehrlichiosis: (HME Ft Chaffee; Quantico, Ft Campbell)
  - Spotted Fever: (Ft Chaffee, Ft Bragg, Botswana)
Typhus Group (TG) Rickettsiae

- Epidemic (louse-borne) Typhus: *Rickettsia prowazekii*
- Murine (flea-borne) Typhus: *R. typhi*

Spotted Fever Group (SFG)

- Rocky Mountain spotted fever: *Rickettsia rickettsii*
- Mediterranean spotted fever: *R. conorii*
- African tick bite fever: *R. africae*
- North Asian/Siberian tick typhus: *R. sibirica*
Rocky Mountain Spotted Fever

- Etiologic agent: *R. rickettsii*

- Reported in USA, Canada, Mexico, Costa Rica, Panama, Columbia, and Brazil.

- Most severe SFG rickettsial disease and is the most commonly fatal tick-borne disease in the United States. Case-fatality rates up to 30% were reported in the pre-antibiotic era but rates have remained between 2-10 % since the 1950’s.

- Recent Brazilian outbreak, 66% of cases were fatal.

- Seroconversions were observed for military units that trained in Arkansas (38%) and more than 40% of these individuals received medical treatment.
Diagnosis of rickettsial disease

• Generally based on clinical presentation and exposure history of a patient.

• Clinical characterizations are sudden onset of fever, primary eschars, severe headache, myalgia, arthralgia, malaise, and skin rashes.

• Differentiating rickettsial diseases from other acute tropical febrile illnesses can be difficult because of the similarities in signs and symptoms.
Currently available laboratory diagnosis of rickettsial diseases

- serodiagnostic assays such as the indirect immunoperoxidase (IIP) assay and indirect immunofluorescent (IFA) tests (weeks)

- nucleic acid detection by PCR amplification of rickettsial genes (days)
General Reaction Scheme

Experimental samples

RNA extraction

Reverse transcription reaction

Hybridization for overnight

Image scanning

Data normalization/analysis

List of up- and down-regulated genes

Replicates/confirmation/reproducibility
Hypothesis

Infection of human monocytes with *R. rickettsii* causes specific mRNA expression pattern. This pattern could be used to distinguish different types of infection and to identify novel genes involved in host response.
Experimental Design

• Human monocytes (THP-1) were infected with *R. rickettsii* for 45 min in 5% CO₂ incubator at 35°C with gentle rotation.

• Non-associated *R. rickettsii* were removed by centrifugation.

• Infected and control THP-1 cells were left in the incubator for additional 1, 4, 8 and 18 h.

• At indicated time, cells were centrifuged, washed with PBS and RNA was extracted with trizol.

• Trizol extracted RNA was further cleaned with a RNeasy kit.
Experimental Design (continued)

• Reverse transcription reaction was carried out using poly dT and Superscript kit in the presence of Cy3-dUTP (for labeling sample RNA) and Cy5-dUTP (for labeling human reference RNA).

• Labeled RNA was purified and hybridized at 42°C for overnight onto a DNA microarray slide with 7680 human genes.

• The fluorescence images were scanned and visualized using GenePix Pro 4.0 (Axon Lab).

• Data was analyzed using web-based analysis software from NCI (http://nciarray.nci.nih.gov).
ScatterPlot log2 Ratios; $r = 0.797$
Up-regulated genes

<table>
<thead>
<tr>
<th>Gene name</th>
<th>fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNRPB</td>
<td>2</td>
</tr>
<tr>
<td>ID1</td>
<td>3</td>
</tr>
<tr>
<td>ZBTB7</td>
<td>4</td>
</tr>
<tr>
<td>BCL2L11</td>
<td>3</td>
</tr>
<tr>
<td>AOX2</td>
<td>6</td>
</tr>
</tbody>
</table>
Down-regulated genes

Gene name

PITX2
CEBPA
SF3A1

fold decrease

-7
Comparison of gene lists from various infectious agents

<table>
<thead>
<tr>
<th>Infectious agents</th>
<th>Orientia tsutsugamushi</th>
<th>R. prowazekii</th>
<th>R. rickettsii</th>
<th>8 others (Nau et. al.)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Orientia tsutsugamushi</strong></td>
<td>20/2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td><strong>R. prowazekii</strong></td>
<td>---</td>
<td>24/7</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td><strong>R. rickettsii</strong></td>
<td>---</td>
<td>---</td>
<td>60/25</td>
<td>2</td>
</tr>
<tr>
<td><strong>8 others</strong></td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>139/62</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data taken from Nau et. al., PNAS, 2002, 99(3), 1503-1508.

<sup>b</sup> Presented as the number of up/down regulated genes.

<sup>c</sup> Numbers represent genes identified in both infectious agents.
Conclusions

• Infection of human monocytes with *R. rickettsii* resulted in 60 up-regulated and 25 down-regulated genes. These affected genes may be important for the design of diagnostic markers.

• Comparison with microarray results from other infectious agents indicated list of unique genes responsive to *R. rickettsii* infection (Poster by Ge et. al, in Diagnostics session ).
Future work

- Confirmation of up- and down-regulated genes.
- Replicates and multiple time points are necessary to identify genes consistently regulated by *R. rickettsii* infection.
- Statistical analysis is needed to better identify genes significantly regulated by *R. rickettsii* infection.
Acknowledgement

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