Temporal relationships of cytokines, chemokines and cellular biomarkers during Human Rhinovirus (HRV) infection in asthmatics

Alex Mann, Alan Bell, Michael Ghebre
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Aims

• Explore the clinical response to HRV infection in a population of mild intermittent Asthma

• Identify baseline immunity markers to HRV induced Asthma worsening during infection

• Investigate the relationship between blood cells, NELF and blood biomarkers/cytokines to clinical responses during infection

• Interpretation of combined results
20 mild asthmatic subjects (GINA 1):
• 13 given HRV and 11 became infected
• 7 given diluent
Study design

20 mild asthmatic subjects (GINA 1):
- 13 given HRV and 11 became infected
- 7 given diluent
- 2 non-evaluable
# Demographics and Baseline characteristics

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Asthma Infected (n=11)</th>
<th>Asthma Uninfected (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>26 ± 4.7</td>
<td>27 ± 9.0</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>9 (82)</td>
<td>6 (86)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>10 (91)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>African/Caribbean</td>
<td>1 (9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>GINA 1</td>
<td>11 (100)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>Lung function - baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEF % predicted</td>
<td>93.7 ± 13.6</td>
<td>91.9 ± 16.9</td>
</tr>
<tr>
<td>FEV1 % predicted</td>
<td>91.0 ± 9.4</td>
<td>91.9 ± 11.8</td>
</tr>
<tr>
<td>Asthma control - Day 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACQ5</td>
<td>0.49 ± 0.23</td>
<td>0.31 ± 0.27</td>
</tr>
<tr>
<td>ACQ6</td>
<td>0.47 ± 0.21</td>
<td>0.3 ± 0.22</td>
</tr>
<tr>
<td>ACQ7</td>
<td>0.58 ± 0.2</td>
<td>0.43 ± 0.31</td>
</tr>
<tr>
<td>Blood eosinophils – Day 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>percentage</td>
<td>4.6 ± 2.3</td>
<td>5 ± 2.7</td>
</tr>
<tr>
<td>count (10^9/L)</td>
<td>0.28 ± 0.15</td>
<td>0.3 ± 0.18</td>
</tr>
<tr>
<td>FeNO ppb - Day 0</td>
<td>48 ± 26</td>
<td>31 ± 11</td>
</tr>
<tr>
<td>Serum total IgE - Day 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin prick test +ve</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Max wheal size (mm)</td>
<td>17 ± 15</td>
<td>18 ± 12</td>
</tr>
<tr>
<td>Number of +ve skin prick tests</td>
<td>2.3 ± 0.79</td>
<td>1.9 ± 0.83</td>
</tr>
<tr>
<td>Maximum wheal SI</td>
<td>2.2 ± 0.36</td>
<td>1.7 ± 0.40</td>
</tr>
</tbody>
</table>

- **Age** – 18-55
- **Gender** – 5 Female/15 Male
- **Atopic** - skin prick test positive
- **Sensitivity** – Reversible + PC<sub>20</sub>&lt;16 mg/ml
- **Asthma Medication** – SABA PRN
- **Smoking history** – non-smoker/&lt; 10 pack yrs
Clinical Endpoint: Virology

Healthy infected subjects have similar magnitude and profile of virus shedding to infected subjects with asthma

Infected subjects with clinically significant asthma worsening (ACQ rise) have similar viral curves to those with no asthma worsening
Clinical endpoints: ACQ change and intercorrelations

Infected subjects with asthma have:
- an early onset of URT symptoms (Day 2,3)
- Delayed LRT symptoms and PEF fall (Day 3,4,5)

Infected subjects with reduced asthma control have:
- Higher URT
- Higher LRT symptoms (Day 4,5,6)
- Greater PEF falls (Day 4,5)
Higher BL blood eosinophils associated with greater increases in ACQ (i.e. greater loss of asthma control)

LRTS for infected subjects with high (red) and low (blue) baseline NELF IL-5

Type 2 immunity (e.g. Eosinophils, IL-5)
Immune Biomarkers: Baseline Susceptibility

Higher BL blood eosinophils associated with greater increases in ACQ (i.e. greater loss of asthma control)

Asthma control:
- Lymphocyte count correlated with ACQ-7 change ($r = 0.75$, $p < 0.05$)

Lung function:
- NELF TNF-alpha and CXCL10 correlated with PEF fall ($r = 0.59$, $p < 0.01$ and $r = 0.64$, $p < 0.05$ respectively).

LRTS for infected subjects with high (red) and low (blue) baseline NELF IL-5

Max daily % fall in PEF for infected subjects with high (red) and low (blue) baseline NELF CXCL10
Nasosorption biomarkers: post inoculation changes

Chemokines for IL-2 activated T cells & NK cells
PEF:
- CXCL19 (MIG), r = -0.75
- CXCL11 (ITAC), r = -0.73
- CXCL10 (IP-10), r = -0.68

LRT:
- CXCL10 (IP-10), r = 0.63
- CXCL11 (ITAC), r = 0.62

Recruitment of immune cells and inflammation, & allergic response
PEF:
- CCL13 (MCP-4), r = -0.69
- CCL5 (RANTES), r = -0.65

LRT:
- CCL8 (MCP-2), r = 0.63
Blood Cell Differentials: Post Inoculation relationships

- Infected subjects had:
  - Greater change in monocytes,
  - Greater change in neutrophils
  - Lower L\%:M\% ratio

- Those with reduced in control had:
  - Greater change in monocytes
  - Greater change in neutrophils
  - Lower L\%:M\% ratio

PEF and LRTS correlated with cells:
- Monocytes: PEF (r=-0.70) & LRTS (r=-0.75)
- Neutrophils: PEF (r=-0.75)
- L\%:M\%: PEF (r-0.63)
Clinical, Cellular, and Biomarker Response to Infection

Recruitment of immune cells and inflammation, & allergic response

Chemokines for IL-2 activated T cells & NK cells

PEF, LRT, and blood cell changes correlated with NELF chemokines/cytokines

<table>
<thead>
<tr>
<th></th>
<th>Mono</th>
<th>Neut</th>
<th>L:M</th>
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<tbody>
<tr>
<td>CCL8</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>CCL13</td>
<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>CCL5</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>CXCL10</td>
<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>CXCL9</td>
<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>CXCL11</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td></td>
<td>X</td>
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<tr>
<td>IFN-α</td>
<td>X</td>
<td>X</td>
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<tr>
<td>IL-10</td>
<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
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Interferon, anti-inflammatory
Clinical, Cellular, and Biomarker Response to Infection

Time course of chemokines and cells in relation to clinical response:

Early phase (Day 2, 3)
- Viral and URT peaks
- Changes in blood cells
- Rise in chemokine markers

Later phase (Day 3, 4, 5, 6)
- LRT and PEF changes
- Migration of immune cells to the respiratory tract
- Rises in chemokine markers in NELF
Summary of findings

• Hypothesis generating challenge study using HRV-16 rhinovirus mild intermittent asthma

• HRV infection induced asthma worsening (ACQ, LRT, PEF)

• Asthma worsening was associated with:
  • Raised baseline type 2 immune pathway markers
  • Post-inoculation changes in
    • blood cells
    • nasal chemokines/cytokines
    • however not type 2
Clinical relevance and future direction

• Within a well studied phenotype different clinical responses to infection associates with baseline characteristics and the subsequent immune response.
• Impacting on T2 pathways prior to viral infection may reduce exacerbation frequency, severity and duration.
• Further studies to better understand phenotypes/endotypes and the interaction of immune pathways in viral infection (Innate, T1, T2, chemokines) is needed.
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- Laura Krizman

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