Synthetic Response of Stimulated Respiratory Epithelium

Modulation by Prednisolone and iKK2 Inhibition

Lucy Bianca Woodman, PhD; Wing Yan Heidi Wan, BSc; Roberta Milone, MSc; Ken Grace, PhD; Ana Sousa, PhD; Rick Williamson, PhD; and Christopher Edward Brightling, PhD, FCCP
## SUPPLEMENTARY FIGURES AND TABLES

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls (n=16)</th>
<th>Asthmatic (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>40 (4)</td>
<td>47 (4)</td>
</tr>
<tr>
<td><strong>Male / Female</strong></td>
<td>6/10</td>
<td>9/8</td>
</tr>
<tr>
<td><strong>Pack Years</strong></td>
<td>0 (0)</td>
<td>2 (1)</td>
</tr>
<tr>
<td><strong>Atopy, %</strong></td>
<td>25%</td>
<td>56%</td>
</tr>
<tr>
<td><strong>IgE (IU/mL)</strong></td>
<td>86 (60)</td>
<td>946 (451)</td>
</tr>
<tr>
<td><strong>ICS, %</strong></td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td><strong>ICS Dose, BDP equivalent</strong></td>
<td>N/A</td>
<td>908 (183)</td>
</tr>
<tr>
<td><strong>OCS, %</strong></td>
<td>N/A</td>
<td>2 (12)</td>
</tr>
<tr>
<td><strong>PC_{20}FEV_{1}, mg/ml †</strong></td>
<td>&gt;16 (0)</td>
<td>0.92 (0.34 – 2.49)</td>
</tr>
<tr>
<td><strong>FEV_{1}/FVC %</strong></td>
<td>85 (2)</td>
<td>75 (2)</td>
</tr>
<tr>
<td><strong>FEV_{1}, % predicted</strong></td>
<td>103 (4)</td>
<td>95 (5)</td>
</tr>
<tr>
<td><strong>Sputum Total Cell Count</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophil, (%)^</td>
<td>N/A</td>
<td>7 (2 – 19)</td>
</tr>
<tr>
<td>Neutrophil, (%)^</td>
<td>N/A</td>
<td>49 (35 – 69)</td>
</tr>
</tbody>
</table>

### e-Table 1: Clinical Characteristics:

Data presented as mean (SEM) unless otherwise indicated, † Geometric mean (95% CI), ^ median (interquartile range), IgE; Immunoglobulin E, IU; International Units, ICS; Inhaled Corticosteroids (µg), BDP; Beclomethasone dipropionate, OCS; Oral corticosteroids, FEV_{1}; Forced expiratory volume in 1 second, PC_{20}; Provocative concentration of methacholine to cause a 20% decrease in FEV_{1}, FVC; Forced vital capacity. ICS dose relates only to those asthmatics taking medication.
Table 2: Constitutive mediator concentration between primary epithelial cell types

<table>
<thead>
<tr>
<th>(pg/ml/10^6 cells)</th>
<th>Constitute Levels (pg/ml/10^6 cells)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nasal Basal (n=6)</td>
<td></td>
</tr>
<tr>
<td>IFN-β *</td>
<td>129.7 (59.0 – 285.8)</td>
<td></td>
</tr>
<tr>
<td>CCL5 *</td>
<td>69.6 (51.1 – 94.9)</td>
<td></td>
</tr>
<tr>
<td>IL-1β *</td>
<td>213 (15.6 – 29.2)</td>
<td></td>
</tr>
<tr>
<td>CXCL8 *</td>
<td>2558.0 (1317 – 4968)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bronchial Basal (n = 17)</td>
<td></td>
</tr>
<tr>
<td>IFN-β *</td>
<td>60.7 (28.8 – 127.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CCL5 *</td>
<td>12.4 (8.9 – 17.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL-1β *</td>
<td>216.1 (81.4 – 573.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CXCL8 *</td>
<td>11205.0 (5367.0 – 23391.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Bronchial Differentiated (n = 21)</td>
<td></td>
</tr>
<tr>
<td>IFN-β *</td>
<td>13.9 (12.5 – 15.1)</td>
<td></td>
</tr>
<tr>
<td>CCL5 *</td>
<td>22.7 (13.3 – 38.5)</td>
<td></td>
</tr>
<tr>
<td>IL-1β *</td>
<td>20.5 (7.5 – 56.2)</td>
<td></td>
</tr>
<tr>
<td>CXCL8 *</td>
<td>12316.0 (8792.0 – 17251.0)</td>
<td></td>
</tr>
</tbody>
</table>
|                   | Constitutive release of mediators over 24h from non-asthmatic and asthmatic nasal, bronchial basal and bronchial differentiated epithelial cells; cytokines are ordered according to the statistical difference of mediators between cell types using ANOVA (p =); significant difference between cell types, with Tukey's post-hoc multiple comparison: ¶ nasal Vs bronchial differentiated, † nasal Vs bronchial basal, † nasal Vs bronchial differentiated, Δ bronchial basal Vs bronchial differentiated. NS: no significant difference.
Constitutive release of mediators over 24h from non-asthmatic or asthmatic nasal basal, bronchial basal and bronchial differentiated epithelial cells; cytokines are ordered according to the statistical difference of mediators between cell types.

c-Table 3. Constitutive mediator concentration between primary epithelial cell types

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Nasal Basal</th>
<th>Bronchial Basal</th>
<th>Bronchial Differentiated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-Asthmatic</td>
<td>Asthmatic</td>
<td>Non-Asthmatic</td>
</tr>
<tr>
<td>IFN-β</td>
<td>86.5 (24.4–306.9)</td>
<td>195.0 (26.1–1458.81)</td>
<td>73.4 (35.0–154.2)</td>
</tr>
<tr>
<td>CCL5</td>
<td>64.9 (23.7–178.2)</td>
<td>74.7 (46.0–121.3)</td>
<td>12.2 (7.26–23.39)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>20.9 (16.3–26.9)</td>
<td>21.7 (6.9–68.4)</td>
<td>287.50 (63.1–1310.0)</td>
</tr>
<tr>
<td>CXCL8</td>
<td>2602.0 (765.9–8841.0)</td>
<td>2514.0 (289.5–21836.0)</td>
<td>17666.0 (5930–52630)</td>
</tr>
<tr>
<td>CCL2</td>
<td>67.2 (18.3–246.5)</td>
<td>58.8 (26.1–132.3)</td>
<td>122.1 (50.6–294.8)</td>
</tr>
<tr>
<td>CCL13</td>
<td>385.6 (128.3–1159.0)</td>
<td>338.1 (177.1–645.3)</td>
<td>854.4 (401.0–1820.0)</td>
</tr>
<tr>
<td>CCL4</td>
<td>35.2 (5.9–208.9)</td>
<td>26.4 (17.0–40.9)</td>
<td>38.8 (7.7–194.5)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>26.8 (13.1–54.7)</td>
<td>26.6 (14.6–48.2)</td>
<td>219.5 (61.7–780.8)</td>
</tr>
<tr>
<td>CCL22</td>
<td>1002.0 (435.5–2307.0)</td>
<td>1127.0 (878.5–1446.0)</td>
<td>1434.0 (692.4–2972.0)</td>
</tr>
<tr>
<td>CCL17</td>
<td>396.1 (156.7–1001.0)</td>
<td>498.2 (212.2–1170.0)</td>
<td>586.6 (286.6–1201.0)</td>
</tr>
<tr>
<td>CCL26</td>
<td>6279.0 (2282.0–17280.0)</td>
<td>6709.0 (3490.0–12896.0)</td>
<td>8414.0 (4393–16116)</td>
</tr>
<tr>
<td>CXCL10</td>
<td>973.2 (410.3–2309.0)</td>
<td>906.6 (572.6–1435.0)</td>
<td>958.7 (441.7–2080.0)</td>
</tr>
<tr>
<td>CCL11</td>
<td>260.2 (53.2–1272.0)</td>
<td>277.9 (193.2–399.7)</td>
<td>321.1 (132.3–779.5)</td>
</tr>
</tbody>
</table>
Table 4: Mediator concentration between either non-asthmatic or asthmatic primary epithelial cell types following poly IC stimulation Fold change (geometric mean; 95% CI)

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Nasal Derived Basal</th>
<th>Bronchial Derived Basal</th>
<th>Bronchial Derived Differentiated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-Asthmatic (n = 3)</td>
<td>Asthmatic (n = 3)</td>
<td>Non-Asthmatic (n = 7)</td>
</tr>
<tr>
<td>IFN-β</td>
<td>1.5 (0.2 - 10.1)</td>
<td>6.7 (1.2 - 38.9)</td>
<td>3.5 (1.1 - 11.9)</td>
</tr>
<tr>
<td>CCL5</td>
<td>399.3 (56.1 - 2846.0)</td>
<td>715.7 (288.8 - 1774.0)</td>
<td>1066.0 (490.0 - 2319.0)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>14.7 (2.0 - 1093.3) #</td>
<td>86.1 (50.8 - 1461.1) #</td>
<td>2.4 (1.0 - 6.0)</td>
</tr>
<tr>
<td>CXCL8</td>
<td>31.4 (4.1 - 241.4)</td>
<td>21.4 (5.9 - 77.4)</td>
<td>3.9 (1.2 - 12.6)</td>
</tr>
<tr>
<td>CCL2</td>
<td>32.7 (4.4 - 242.8)</td>
<td>84.3 (37.1 - 191.4)</td>
<td>6.5 (0.9 - 45.9)</td>
</tr>
<tr>
<td>CCL13</td>
<td>14.1 (3.3 - 61.0)</td>
<td>23.6 (21.1 - 48.6)</td>
<td>3.5 (0.9 - 14.5)</td>
</tr>
<tr>
<td>CCL4</td>
<td>279.7 (51.0 - 1530.0)</td>
<td>841.0 (491.2 - 1440.0)</td>
<td>133.4 (19.0 - 940.4)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>52.3 (10.4 - 263.3) #</td>
<td>403.8 (225.8 - 722.0) #</td>
<td>8.7 (4.6 - 16.4)</td>
</tr>
<tr>
<td>CCL22</td>
<td>19.5 (3.5 - 109.3)</td>
<td>32.0 (21.1 - 48.6)</td>
<td>5.0 (0.8 - 29.8)</td>
</tr>
<tr>
<td>CCL17</td>
<td>46.8 (3.4 - 642.6)</td>
<td>90.7 (31.6 - 260.2)</td>
<td>7.4 (0.9 - 60.6)</td>
</tr>
<tr>
<td>CCL26</td>
<td>86.9 (20.8 - 363.2)</td>
<td>199.0 (12.2 - 33359.0)</td>
<td>2.0 (0.8 - 4.9)</td>
</tr>
<tr>
<td>CXCL10</td>
<td>500.9 (15.6 - 16117.0)</td>
<td>282.6 (95.3 - 838.2)</td>
<td>115.7 (9.6 - 1393.0)</td>
</tr>
<tr>
<td>CCL11</td>
<td>26.2 (3.5 - 193.5)</td>
<td>33.2 (10.0 - 110.0)</td>
<td>9.2 (2.1 - 41.1)</td>
</tr>
</tbody>
</table>

Note: # Significant difference compared to constitutive levels. **p<0.001**, *p<0.01*, †p<0.05 compared to constitutive levels. ©2013 American College of Chest Physicians. See online for more details.
### e-Table 5: Mediator concentration between either non-asthmatic or asthmatic primary epithelial cell types following IL-1β stimulation

Fold change (geometric mean; 95% CI) over constitutive levels after stimulation for 24h, coloured boxes;

- Light green: *p*≤0.05
- Orange: *p*≤0.01
- Light yellow: *p*≤0.001 compared to constitutive levels.

# Significant difference between non-asthmatic & asthmatic donors.
Table 6: Mediator concentration between either non-asthmatic or asthmatic primary epithelial cell types following IL-1β & IFN-γ stimulation Fold change (geometric mean; 95% CI) over constitutive levels after stimulation for 24h, coloured boxes: p<0.05 compared to constitutive levels after stimulation for 24h, primary donors.

<table>
<thead>
<tr>
<th></th>
<th>Nasal Derived</th>
<th>Bronchial Derived</th>
<th>Bronchial Derived</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal (n = 3)</td>
<td>Basal (n = 7)</td>
<td>Differentiated (n = 10)</td>
</tr>
<tr>
<td></td>
<td>Non-Asthmatic</td>
<td>Asthmatic (n = 7)</td>
<td>Non-Asthmatic (n = 10)</td>
</tr>
<tr>
<td>Non-Asthmatic</td>
<td>0.7 (0.2 - 2.4)</td>
<td>0.7 (0.1 - 3.7)</td>
<td>0.9 (0.5 - 1.5)</td>
</tr>
<tr>
<td>IFN-β</td>
<td>1.0 (0.8 - 1.3)</td>
<td>1.0 (1.0 - 1.0)</td>
<td>0.8 (0.6 - 1.1)</td>
</tr>
<tr>
<td>CCL5</td>
<td>3.7 (0.6 - 24.6)</td>
<td>17.7 (1.5 - 209.3)</td>
<td>41.5 (10.0 - 172.7)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CXCL8</td>
<td>22.6 (1.9 - 274.6)</td>
<td>41.2 (2.3 - 747.3)</td>
<td>3.9 (1.3 - 11.6)</td>
</tr>
<tr>
<td></td>
<td>4.7 (2.1 - 10.7)</td>
<td>2.3 (0.5 - 9.7)</td>
<td>1.7 (0.9 - 3.2)</td>
</tr>
<tr>
<td>CCL2</td>
<td>55.7 (10.1 - 305.8)</td>
<td>27.4 (2.0 - 367.5)</td>
<td>95.9 (11.4 - 310.9)</td>
</tr>
<tr>
<td>CCL13</td>
<td>34.4 (8.1 - 145.3)</td>
<td>10.9 (1.7 - 68.4)</td>
<td>4.9 (1.6 - 15.3)</td>
</tr>
<tr>
<td>CCL4</td>
<td>161.8 (50.7 - 516.3)</td>
<td>47.7 (15.2 - 149.2)</td>
<td>44.3 (4.1 - 478.3)</td>
</tr>
<tr>
<td></td>
<td>41.5 (11.3 - 152.1)</td>
<td>295.1 (8.4 - 10360.0)</td>
<td>274 (15.7 - 47.8)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>4.7 (2.9 - 7.5)</td>
<td>6.1 (1.7 - 22.0)</td>
<td>3.27 (2.4 - 4.5)</td>
</tr>
<tr>
<td></td>
<td>5.4 (3.2 - 9.3)</td>
<td>21.1 (0.3 - 1352.0)</td>
<td>2.6 (1.5 - 4.5)</td>
</tr>
<tr>
<td>CCL22</td>
<td>25.3 (8.1 - 145.3)</td>
<td>8.1 (1.9 - 34.8)</td>
<td>7.2 (1.7 - 29.8)</td>
</tr>
<tr>
<td>CCL17</td>
<td>62.8 (18.6 - 211.4)</td>
<td>14.0 (1.5 - 126.8)</td>
<td>14.0 (3.1 - 63.5)</td>
</tr>
<tr>
<td>CCL26</td>
<td>50.5 (20.9 - 119.5)</td>
<td>15.9 (2.1 - 18.3)</td>
<td>3.5 (1.9 - 6.5)</td>
</tr>
<tr>
<td>CXCL10</td>
<td>637.1 (68.4 - 5935.0)</td>
<td>239.8 (10.9 - 5268.0)</td>
<td>178.7 (64.7 - 494.1)</td>
</tr>
<tr>
<td></td>
<td>503.6 (82.3 - 3082.0)</td>
<td>994.3 (60.1 - 16439.0)</td>
<td>504.5 (163.1 - 1560.0)</td>
</tr>
<tr>
<td>CCL11</td>
<td>57.8 (23.4 - 142.7)</td>
<td>16.2 (1.8 - 146.1)</td>
<td>15.4 (5.3 - 44.3)</td>
</tr>
<tr>
<td></td>
<td>12.5 (5.7 - 27.5)</td>
<td>19.5 (7.1 - 53.1)</td>
<td>38.9 (5.8 - 261.9)</td>
</tr>
</tbody>
</table>
Table 7: The ratio of the mean IKK2 over Prednisolone efficacy for each stimulus on basal epithelial cells.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Stimuli</th>
<th>Ratio (IKK2i / Pred)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL11</td>
<td>IL-1β</td>
<td>0.67</td>
<td>0.13</td>
</tr>
<tr>
<td>CCL11</td>
<td>IL-1B/IFN-γ</td>
<td>0.52</td>
<td>0.03</td>
</tr>
<tr>
<td>CCL11</td>
<td>PolyIC</td>
<td>0.25</td>
<td>0.00</td>
</tr>
<tr>
<td>CCL26</td>
<td>IL-1β</td>
<td>0.23</td>
<td>0.02</td>
</tr>
<tr>
<td>CCL26</td>
<td>IL-1B/IFN-γ</td>
<td>0.31</td>
<td>0.07</td>
</tr>
<tr>
<td>CCL26</td>
<td>PolyIC</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>IFN-β</td>
<td>IL-1β</td>
<td>1.13</td>
<td>0.82</td>
</tr>
<tr>
<td>IFN-β</td>
<td>IL-1B/IFN-γ</td>
<td>1.10</td>
<td>0.86</td>
</tr>
<tr>
<td>IFN-β</td>
<td>PolyIC</td>
<td>0.38</td>
<td>0.07</td>
</tr>
<tr>
<td>CCL8</td>
<td>IL-1β</td>
<td>0.28</td>
<td>0.00</td>
</tr>
<tr>
<td>CCL8</td>
<td>IL-1B/IFN-γ</td>
<td>0.24</td>
<td>0.00</td>
</tr>
<tr>
<td>CCL8</td>
<td>PolyIC</td>
<td>0.38</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL13</td>
<td>IL-1β</td>
<td>0.51</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL13</td>
<td>IL-1B/IFN-γ</td>
<td>0.34</td>
<td>0.00</td>
</tr>
<tr>
<td>IL13</td>
<td>PolyIC</td>
<td>0.32</td>
<td>0.00</td>
</tr>
<tr>
<td>IL-1β</td>
<td>PolyIC</td>
<td>0.83</td>
<td>0.02</td>
</tr>
<tr>
<td>IL8</td>
<td>IL-1β</td>
<td>0.27</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL8</td>
<td>IL-1B/IFN-γ</td>
<td>0.16</td>
<td>0.00</td>
</tr>
<tr>
<td>IL8</td>
<td>PolyIC</td>
<td>0.22</td>
<td>0.00</td>
</tr>
<tr>
<td>CXCL10</td>
<td>IL-1β</td>
<td>0.38</td>
<td>0.15</td>
</tr>
<tr>
<td>CXCL10</td>
<td>IL-1B/IFN-γ</td>
<td>0.58</td>
<td>0.45</td>
</tr>
<tr>
<td>CXCL10</td>
<td>PolyIC</td>
<td>0.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CCL2</td>
<td>IL-1β stim</td>
<td>0.51</td>
<td>0.04</td>
</tr>
<tr>
<td>CCL2</td>
<td>IL-1B/IFN-γ stim</td>
<td>0.09</td>
<td>0.00</td>
</tr>
<tr>
<td>CCL2</td>
<td>PolyIC stim</td>
<td>0.29</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CCL13</td>
<td>IL-1β stim</td>
<td>0.33</td>
<td>0.08</td>
</tr>
<tr>
<td>CCL13</td>
<td>IL-1B/IFN-γ stim</td>
<td>0.44</td>
<td>0.22</td>
</tr>
<tr>
<td>CCL13</td>
<td>PolyIC stim</td>
<td>0.40</td>
<td>0.20</td>
</tr>
<tr>
<td>CCL22</td>
<td>IL-1β stim</td>
<td>0.44</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CCL22</td>
<td>IL-1B/IFN-γ stim</td>
<td>0.48</td>
<td>0.01</td>
</tr>
<tr>
<td>CCL22</td>
<td>PolyIC stim</td>
<td>0.39</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CCL4</td>
<td>IL-1β stim</td>
<td>0.24</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CCL4</td>
<td>IL-1B/IFN-γ stim</td>
<td>0.27</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CCL4</td>
<td>PolyIC stim</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>CCL5</td>
<td>IL-1β stim</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>CCL5</td>
<td>IL-1B/IFN-γ stim</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>CCL5</td>
<td>PolyIC stim</td>
<td>0.13</td>
<td>0.00</td>
</tr>
<tr>
<td>CCL17</td>
<td>IL-1β stim</td>
<td>0.76</td>
<td>0.52</td>
</tr>
<tr>
<td>CCL17</td>
<td>IL-1B/IFN-γ stim</td>
<td>0.40</td>
<td>0.05</td>
</tr>
<tr>
<td>CCL17</td>
<td>PolyIC stim</td>
<td>0.36</td>
<td>0.04</td>
</tr>
<tr>
<td>TNF-α</td>
<td>IL-1β stim</td>
<td>0.31</td>
<td>0.00</td>
</tr>
<tr>
<td>TNF-α</td>
<td>IL-1B/IFN-γ stim</td>
<td>0.50</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TNF-α</td>
<td>PolyIC stim</td>
<td>0.18</td>
<td>0.00</td>
</tr>
</tbody>
</table>
e-Table 8: The ratio of the mean IKK2 over Prednisolone response for the donors who responded to the poly IC stimulus and corresponding p-value.

Data was analysed on the log10 scale. Data was excluded from the analysis where polyIC-stimulated cytokine release was <3-fold constitutive for a particular donor. A linear model was then fitted to the remaining data to assess whether there was a significant difference in the mean response between the IKK2 and Prednisolone groups. This model included the poly IC stimulus response as a covariate.  

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Ratio (IKK2 / Pred)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL11</td>
<td>0.33</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>CCL26</td>
<td>0.41</td>
<td>0.04</td>
</tr>
<tr>
<td>IFN-β</td>
<td>0.46</td>
<td>0.21</td>
</tr>
<tr>
<td>CXCL8</td>
<td>0.88</td>
<td>0.70</td>
</tr>
<tr>
<td>IL13</td>
<td>0.81</td>
<td>0.77</td>
</tr>
<tr>
<td>CXCL10</td>
<td>0.11</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>CCL2</td>
<td>0.15</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>CCL22</td>
<td>0.46</td>
<td>0.02</td>
</tr>
<tr>
<td>CCL4</td>
<td>0.12</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>CCL5</td>
<td>0.17</td>
<td>0.00</td>
</tr>
<tr>
<td>CCL17</td>
<td>0.38</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.38</td>
<td>0.14</td>
</tr>
</tbody>
</table>
Legends

e-Figure 1: Constitutive mediator concentration by primary epithelial cell types between asthmatics and non-asthmatics. Constitutive expression after 24h of mediators from either non-asthmatic (white bars) or asthmatic (black bars), nasal basal (Nb), bronchial basal (Bb), bronchial differentiated (Bd) epithelial cells. Cytokines are ordered according to the statistical difference (*p≤0.05) (n=6 nasal basal, n=17 bronchial basal, n=22 bronchial differentiated non-asthmatic/asthmatic cells).

e-Figure 2: Poly IC induces increased mediator expression. Poly IC (0.5-25µg/ml) & Poly dIdC (0.5-25µg/ml) stimulated (A) CCL4 and (B) CCL26 expression after 24h from nasal basal epithelial cells (n=6) expressed as fold change over constitutive levels (* p<0.05)

e-Figure 3: IKK2i significantly inhibits stimulated mediator production across a wide range of mediators. IKK2i significantly inhibits (A,B) Poly IC or (C,D) IL-1β & IFN-γ mediated (A,C) CXCL8 or (B,D) IL-2 expression from non-asthmatic and asthmatic bronchial basal epithelial cells (n=6) after 24h. Fold change compared to stimulated levels (p<0.05).

e-Figure 4: IKK2i significantly inhibits stimulated mediator productions across a wide range of cells. IKK2i significantly inhibits (A) CXCL10, (B) CXCL8 and (C) CCL4 expression from non-asthmatic and asthmatic bronchial and differentiated epithelial cells (n=17) after 24h expressed as pg/ml/10^6 cells (geometric mean; 95% CI) (* p<0.05, ** p<0.01, ***p<0.001).
e-Figure 1: Constitutive mediator concentration between primary epithelial cell types
e-Figure 2: Poly IC induces increased mediator expression.
e-Figure 3: IKK2i significantly inhibits stimulated mediator production across a wide range of mediators.
e-Figure 4: IKK2i significantly inhibits stimulated mediator productions across a wide range of cells.