SUPPLEMENT

EOSINOPHIL CYSTEINYL LEUKOTRIENE SYNTHESIS MEDIATED BY EXOGENOUS SECRETED PHOSPHOLIPASE A₂ GROUP X

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**SUPPLEMENTAL RESULTS**

**Supplemental Table 1:** Transfected HEK293T cell-based LPS assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Polymixin</th>
<th>IL-8 pg/ml (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>N</td>
<td>105.6 (6.6)</td>
</tr>
<tr>
<td>LPS</td>
<td>N</td>
<td>&gt;3937.5</td>
</tr>
<tr>
<td>sPLA₂-X (100 nM)</td>
<td>N</td>
<td>122.6 (19.8)</td>
</tr>
<tr>
<td>Untreated</td>
<td>Y</td>
<td>107.2 (9.5)</td>
</tr>
<tr>
<td>LPS</td>
<td>Y</td>
<td>105.5 (13.9)</td>
</tr>
<tr>
<td>sPLA₂-X (100 nM)</td>
<td>Y</td>
<td>102.2 (15.6)</td>
</tr>
</tbody>
</table>

* Polymixin B binds to LPS and eliminates IL-8 expression.
**Supplemental Table 2**: Selectivity of sPLA₂-X inhibitor (ROC-0929) and structurally similar control inhibitor (ROC-0428) for the 9 mammalian sPLA2s [IC50 (nM)*].

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>sPLA₂</th>
<th>ROC-0929</th>
<th>ROC-0428</th>
</tr>
</thead>
<tbody>
<tr>
<td>hGIB</td>
<td>&gt;1600</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>hGIIA</td>
<td>&gt;1600</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>hGIID**</td>
<td>700±200</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>hGIIE</td>
<td>&gt;1600</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>hGIIF</td>
<td>&gt;1600</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>hGIIF</td>
<td>&gt;1600</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>hGV</td>
<td>&gt;1600</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>hGX</td>
<td>20±10</td>
<td>6600±900</td>
<td></td>
</tr>
<tr>
<td>hGXIIA</td>
<td>&gt;1600</td>
<td>nd</td>
<td></td>
</tr>
</tbody>
</table>

*IC50 values obtained using fluorometric assay with pyrene-labeled phosphatidylglycerol as substrate.

**IC50 value obtained using radiolabeled *E. coli* membrane assay.
**Supplemental Table 3:** Selectivity of inhibitors for recombinant human sPLA$_2$-X (9.7 - 22.8 nM)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>ROC-0929</th>
<th>ROC-0428</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration</strong></td>
<td>667 nM</td>
<td>33 nM</td>
</tr>
<tr>
<td><strong>% Inh (SD)</strong></td>
<td>85.9 (8.3)</td>
<td>43.0 (7.4)</td>
</tr>
</tbody>
</table>
SUPPLEMENTAL FIGURE LEGENDS:

Supplemental Figure 1. Effect of cPLA\(_{2}\alpha\) inhibitors on sPLA\(_{2}\)-V-mediated eosinophil CysLT synthesis. A, Treatment of eosinophils with 100 nM exogenous sPLA\(_{2}\)-V for 20 min (black bar) increased CysLT synthesis over buffer control (Control, *P=0.06); in agreement with prior evidence, the cPLA\(_{2}\alpha\) inhibitors Pyr-2 and Wyeth-2 failed to significantly decrease sPLA\(_{2}\)-V-mediated CysLT synthesis by eosinophils. B, Because of the broad range of basal and stimulated CysLT production from the 3 eosinophil donors used for these studies, plots of the individual data are shown to confirm that the same result of an increase in CysLT synthesis that was not suppressed by cPLA\(_{2}\alpha\) inhibitors occurred in all the subjects.

Supplemental Figure 2. Additional details of the activation of CysLT synthesis in fMLP stimulated eosinophils by sPLA\(_{2}\)-X. A, Individual eosinophil donor results for the CysLT production in eosinophils mediated by fMLP (10 nM) alone, or with the addition of sPLA\(_{2}\)-X. Exogenous sPLA\(_{2}\)-X at concentrations of 10 nM and 100 nM increased CysLT synthesis over fMLP alone in all subjects. B, Similarly, eosinophils from each donor treated with a higher concentration of fMLP (100 nM) made significant quantities of CysLTs that was further increased in all subjects by the addition of sPLA\(_{2}\)-X at concentrations of 10 nM and 100 nM.

Supplemental Figure 3. Full analysis of the exogenous sPLA\(_{2}\)-X-mediated generation of lysophospholipid species by eosinophils. A-E, Treatment of eosinophils with sPLA\(_{2}\)-X (100 nM) initiated the generation lysophosphatidylcholine (LysoPC) species (A), lysophosphatidylethanolamine (LysoPE) species (B), lysophosphatidylserine (lysoPS) species (C), lysophosphatidylinositol (LysoPI) species (D), and lysophosphatidylglycerol (LysoPG) species (E) relative to control conditions (white bar) and relative to eosinophils treated with fMLP (100 nM). F, Neither sPLA\(_{2}\)-X or fMLP caused significant release of lysophosphatidic acid (LysoPA) species by eosinophils. *P≤0.01 and †P≤0.05 overall. ‡P≤0.01 and §P≤0.05 versus fMLP.

Supplemental Figure 4. Exogenous sPLA\(_{2}\)-X-mediated generation of plasmenyl lysophospholipids and lyso-platelet activating factor by eosinophils. A-C, Treatment of eosinophils with sPLA\(_{2}\)-X (100 nM) initiated the generation of plasmenyl LysoPC species (A), plasmenyl LysoPE species (B), and lyso-platelet activating factor (LysoPAF) species (C) relative to control conditions (white bar) and relative to eosinophils treated with fMLP (100 nM). *P≤0.01 and †P≤0.05 overall. ‡P≤0.01 and §P≤0.05 versus fMLP.

Supplemental Figure 5. Control images for confocal microscopy. The first column of images (A) shows eosinophils that have been stimulated with fMLP, but have not been labeled with either a primary or secondary antibody. The second column (B) shows eosinophils that have been labeled with a Cy3-anti-rabbit antibody, but have not been treated with a primary antibody. The third column (C) shows eosinophils that have been labeled with rabbit IgG at the same concentration as the antibodies used to detect cPLA\(_{2}\) and 5-LO, and labeled with a Cy3-anti-rabbit antibody.
Supplemental Figure 1

**A**

![Bar chart A](chart.png)

**B**

![Bar chart B](chart.png)
Supplemental Figure 2

A

B

CysLTs pg/10^6 eosinophils

Control
IMLP 10 nM
IMLP 10 nM + sPLA2-X 10 nM
IMLP 10 nM + sPLA2-X 100 nM

Subject 1
Subject 2
Subject 3
Subject 4

CysLTs pg/10^6 eosinophils

Control
IMLP 100 nM
IMLP 100 nM + sPLA2-X 10 nM
IMLP 100 nM + sPLA2-X 100 nM

Subject 1
Subject 2
Subject 3
Subject 4
Supplemental Figure 3
Supplemental Figure 4

A. Plasmenyl LysoPC Species

B. Plasmenyl LysoPE Species

C. LysoPAF Species
Supplemental Figure 5

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cy3 Channel</th>
<th>Merge with DIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No 1\textsuperscript{o} or Cy3</td>
<td>Black</td>
<td></td>
</tr>
<tr>
<td>No 1\textsuperscript{o} w/Cy3 Ab</td>
<td>Black</td>
<td></td>
</tr>
<tr>
<td>Rabbit IgG w/Cy3</td>
<td>Black</td>
<td></td>
</tr>
</tbody>
</table>