**Background**

- Macrophages are major propagators of inflammation during cardiac homeostasis and disease.
- The NLRP3 inflammasome is primed and activated in response to mitochondrial damage, oxidative stress, and other stimuli associated with cardiac insults.
- Cytochrome P450 (CYP) epoxide hydrolases metabolize n-3 and n-6 polyunsaturated fatty acids (PUFA). The resultant PUFA epoxides can be hydrolyzed to vicinal diols by soluble epoxide hydrolase (sEH).
- CYP-derived oxylipins 19,20-epoxydocosapentaenoic acid (19,20-EDP) and 12,13-dihydroxyoctadecanoic acid (12,13-DiHOME) have beneficial and detrimental effects on cardiomyocyte mitochondrial quality, respectively.
  - GPR120 is an anti-inflammatory and NLRP3-inhibiting PUFA receptor. Upstream effects of LPS/oxylipins on GPR120 are poorly characterized.
  - We hypothesize that CYP-derived oxylipins differentially modulate macrophage mitochondria to impact pro-inflammatory signaling.

**Methods**

**Murine RAW 264.7 macrophage cell line**

- Treat cardiomyocytes with macrophage-conditioned media (MCM) for 6h.

**Human THP1-derived macrophage cell line**

- Treat cardiomyocytes with macrophage-conditioned media (MCM) for 6h.

**RESULTS: GPR120 REGULATION IN MACROPHAGES**

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<tr>
<th>RAW 264.7</th>
<th>LPS/EDP/DiHOME</th>
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<tr>
<td><strong>GPR120</strong></td>
<td><strong>Beta-actin</strong></td>
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**RESULTS: NLRP3 INFLAMMASOME AND M1 POLARIZATION**

**Figure 1.** Effect of oxylipins on NLRP3 inflammasome in THP1-derived macrophages. THP1-derived macrophages were treated with 100 ng/mL LPS and 19,20-EDP or 12,13-DiHOME for 24 hours before immunoblotting. NLRP3 and pro-caspase-1 levels were compared to a mean of “1.0” using one-sample t-test to test significance (n=4). *p<0.05, **p<0.01.

**Figure 2.** M1 polarization, NLRP3 inflammasome protein expression, and GPR120 in THP1-derived macrophages. THP1-derived macrophages were treated with pro-inflammatory M1 polarization stimuli (LPS and IFNγ) with or without 19,20-EDP or 12,13-DiHOME for 24 hours before immunoblotting. NLRP3 and pro-caspase-1 showed no significant differences (data not shown). Mean pro-IL-1β (n=4) and GPR120 (n=3) levels were compared to a mean of “1.0” using one-sample t-test to test significance. *p<0.05, **p<0.01.

**Figure 3.** Macrophage GPR120 localization in response to LPS. RAW 264.7 or THP1-derived macrophages were treated with 100 ng/mL LPS for the indicated times. Whole cell lysates and reconstituted insoluble pellets (crude membrane fraction) were immunoblotted. GPR120 internalization may be inferred as increasing GPR120 in lysate with a simultaneous decrease in crude membrane fraction. GAPDH was used as a soluble protein control. One-sample t-test comparing to a mean value of “1.0” was used to test significant differences in RAW 264.7 cells (n=3) but not THP-1 (n=2). *p<0.05, **p<0.01.

**Figure 4.** Effect of macrophage-conditioned media (MCM) on NLRP3 inflammasome protein expression in isolated rat neonate cardiomyocytes. RAW 264.7 cells were pre-treated for 1 hour with oxylipins prior to 6 hour stimulation with LPS. Following treatment, CM was frozen and used to treat isolated rat neonate cardiomyocytes (DM). For the last 30 mins, DHA was a positive control for inflammasome inhibition. Groups were compared to a mean of “1.0” used to test significance (n=3). *p<0.05, **p<0.01.

**Figure 5.** Proposed model for CYP-derived oxylipin involvement in macrophage NLRP3 inflammasome activity in THP1-derived macrophages.

- 12,13-DiHOME and 19,20-EDP exacerbatate NLRP3 inflammasome activation in THP1-derived macrophages.
- Contrarily, 19,20-EDP pre-treatment in murine RAW 264.7 macrophages inhibits NLRP3 induction by MCM in cardiomyocytes.
- LPS induces GPR120 internalization in RAW 264.7 and THP1-derived macrophages.
- M1 polarization and 12,13-DiHOME downregulates GPR120 in THP1 macrophages.
- Connection between effects of LPS/oxylipins on GPR120 and mitochondrial quality (ΔΨm, morphology, ROS, cytosolic mtDNA) and NLRP3 inflammasome activity in macrophages remains to be established.

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**References**


**Figure 6.** Proposed model for CYT-derived oxylipin involvement in macrophage NLRP3 inflammasome activity in THP1-derived macrophages.