Inhaled Air Pollution Particulate Matter in Alveolar Macrophages Alters local pro-inflammatory Cytokine and peripheral IFNγ Production in Response to *Mycobacterium tuberculosis*
Urban Air Pollution – A Global Phenomenon
What is Particulate Matter (PM)?

Urban particulate matter (PM): a carbon core coated with range of chemical species including reactive transition metals and organic hydrocarbons.

1 µm is 1/1000 millimeter
Global Mortality from Urban Outdoor and Household Air Pollution

In 2012 WHO reported 1 of 8 global deaths – as a result of air pollution exposure.

...air pollution is now the world’s largest single environmental health risk.

1600 cities worldwide are reporting air pollution levels

3.7 million deaths attributable to urban / outdoor air pollution

4.3 million deaths attributable to household / indoor air pollution

http://www.who.int/phe/health_topics/outdoorair/databases/en/

http://www.humanosphere.org/global-health/2017/03/
Increased Risk of TB development and Susceptibility to *M.tb* infection from Inhalation Exposure to Air Pollutants

- *Mycobacterium tuberculosis* (*M.tb*), that causes tuberculosis (TB) infects 10.4 million people and causes 1.4 million deaths annually.

- While most *M.tb* infection remain asymptomatic, immunosuppressive states of the host increase the risk of TB development.

- Exposure to tobacco smoke and household air pollution significantly increases risk of acquisition of *M.tb* infection and TB development.

- Recent studies indicate that urban outdoor air pollution exposure may increase risk of TB development and mortality of TB patients during TB therapy.
Urban Ambient PM \textit{in vitro} Exposure Effects on \textit{M. tb} Responses in A549 Respiratory Epithelial Cells

PM dose-dependent
- suppression of \textit{M. tb}-induced HBD-2 mRNA and protein expression
- loss of \textit{M. tb} growth control
- induction of cellular senescence (SA-\(\beta\)-gal)


Human Subject and PM Studies

Overall Hypothesis
Exposure to urban ambient air pollution particulate matter (PM) modifies human antimycobacterial immunity (impairing innate and adaptive effector cell functions).

Study Population

- Recruited 32 healthy, HIV-1 seronegative, nonsmoking (urine cotinine-negative) volunteers (female n= 11, male n=21)
- Residents of Iztapalapa (minimum 1 yr. prior to studies)
- Median age 28 yr. (min. 21, max. 46 yr.)
- 30 IGRA-, 2 IGRA+ (1 TST+) (TST+ 7/32 subjects)

Study Material

- Bronchoalveolar cells (BAC)
- PBMC

<table>
<thead>
<tr>
<th>Bronchoalveolar cells (BAC)</th>
<th>Alveolar Macrophages (%)</th>
<th>Alveolar Lymphocytes (%)</th>
<th>Alveolar Neutrophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>91.1</td>
<td>8.9</td>
<td>Scanty</td>
</tr>
<tr>
<td>Maximum</td>
<td>99.3</td>
<td>23.7</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>76.3</td>
<td>0.7</td>
<td></td>
</tr>
</tbody>
</table>
Iztapalapa PM Collection Site

$\text{PM}_{2.5}$ diameter $< 2.5 \mu\text{m}$

$\text{PM}_{10}$ diameter $< 10 \mu\text{m}$
PM Effects on Phagocytosis of *M. tb* in AM

<table>
<thead>
<tr>
<th>MOI</th>
<th>No PM</th>
<th>Cold dry</th>
<th>Warm dry</th>
<th>Rainy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

PM (1µg/ml) vs PM (5µg/ml)
PM-Load in Alveolar Macrophages from Real-World Air Pollution-exposed Healthy Persons
PM Content in Alveolar Macrophages from Air Pollution-exposed Persons from inhalation

Proportion (%) of total alveolar macrophage area occupied by PM

<table>
<thead>
<tr>
<th>Subject</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>Std error</th>
</tr>
</thead>
<tbody>
<tr>
<td>027M</td>
<td>1.79</td>
<td>0.08</td>
<td>5.34</td>
<td>0.38</td>
</tr>
<tr>
<td>022H</td>
<td>0.87</td>
<td>0.13</td>
<td>6.66</td>
<td>0.28</td>
</tr>
<tr>
<td>023M</td>
<td>4.45</td>
<td>0.13</td>
<td>14.05</td>
<td>1.04</td>
</tr>
<tr>
<td>025H</td>
<td>3.36</td>
<td>0.30</td>
<td>21.42</td>
<td>1.23</td>
</tr>
</tbody>
</table>
Wright’s-stained cytospin preparations of BAC from 30 participants were examined to assess proportions of AM containing PM (AM% with PM) and the mean and minimum and maximum areas of the total AM area occupied by PM (PM load per AM). 1000x-magnified digital bright field microscopy – and Image J analysis.
PM Effects on cytokine Production in BAC and PBMC

Open symbol BAC with lower %AM with PM
Red symbol BAC with higher %AM with PM

Open symbol BAC with lower PM load per AM
Blue symbol BAC with higher PM load per AM
Correlations between AM PM Burden and Cytokine Responses of BAC

- **NO PM**
  - TNF-α in BAC
    - $r = -0.6333$
    - $P = 0.0380$
  - IL-1β in BAC
    - $r = -0.7866$
    - $P = 0.0078$

- **PM (10 µg/ml)**
  - TNF-α in BAC
    - $r = -0.6667$
    - $P = 0.0294$
  - IL-1β in BAC
    - $r = -0.7000$
    - $P = 0.0216$

- **M.tb (MOI 1)**
  - PM (10µg/ml)
    - $r = -0.6833$
    - $P = 0.0262$
  - $r = -0.6167$
    - $P = 0.0429$

- **M.tb (MOI 10)**
  - PM (10µg/ml)
    - $r = -0.7000$
    - $P = 0.0216$
  - $r = -0.6333$
    - $P = 0.0380$
  - $r = -0.6167$
    - $P = 0.0429$
Correlations between AM PM Burden and IFNγ Responses in BAC and PBMC
Conclusions

- *M. tb* phagocytosis is not significantly altered by PM-exposure of AM implying that subsequently observed host immune modulations are not due to differences in cellular *M. tb* uptake.
- High level of PM burden was observed in freshly isolated BAC.
- PM load in AM is inversely correlated with *M. tb*-induced IL-1β and TNF-α-production in BAC – whereas such correlations cannot be found in PBMC.
- Interestingly, PM load in AM in response to PPD is inversely correlated with the IFN-γ production in PBMC. Since peripheral blood IFN-γ responses are important biomarkers of *M. tb* infection, PM exposure may affect IFN-γ readouts following TB vaccination and *M. tb* immunodiagnostics.
- The observed suppressive effects of PM on human local (BAC) and systemic (PBMC) antimycobacterial immune responses to *M. tb* may lead to increased susceptibility to pulmonary infection.
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