Pulmonary Health Effects I: Inside the lungs

University of California Los Angeles
University of Southern California
Michigan State University
University of California Irvine
University of Wisconsin-Madison
Prevalence of Allergic Disease Since the Industrial Revolution

Saxon et al
The Impact of Air Pollution on Asthma in Children

Asthma Prevalence by Age
United States, 1980-1994

The Impact of Particulate Matter on Bronchitis Prevalence Among Children with Asthma, Southern California, 1993

Source: National Health Survey, 1980-1994

Source: Southern California Environmental Health Sciences Center, 2007
1. Why are pollutant particles dangerous?

2. Is it the particles themselves or their chemical components that cause the damage?

3. What are the potential mechanisms?
Diesel Exhaust

Soot and Metallic Ash Particles

Air Dilution and Cooling

New Particle Formation and Growth (Homogenous Nucleation Adsorption/Condensation)

Soot Particle with Adsorbed/Condensed Layers of Hydrocarbon and Sulfate

Hot Exhaust

Carbon core

Diesel Exhaust Particles

Organic chemical & metal coating

DEP as a model particulate pollutant

Diesel Exhaust
Mouse Macrophage Studies

1. DEP toxicity is significantly reduced after the removal of organic chemicals.

2. Antioxidant, NAC, could effectively inhibit DEP-induced oxidative stress as determined by the level of intracellular reactive oxygen radicals.

3. NAC could effectively inhibit DEP toxicity.

The effect of antioxidant on DEP toxicity

<table>
<thead>
<tr>
<th>DEP Extract (µg/ml)</th>
<th>Dead Cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>− NAC</td>
<td>+ NAC</td>
</tr>
<tr>
<td>None</td>
<td>3.8</td>
</tr>
<tr>
<td>30</td>
<td>10.3</td>
</tr>
<tr>
<td>60</td>
<td>42.8</td>
</tr>
<tr>
<td>100</td>
<td>74.7</td>
</tr>
</tbody>
</table>
Human Bronchial Epithelial Studies

- Organic extract of DEP induced dose- and time-dependent toxicity in human bronchial epithelial cells

- Polycyclic aromatic hydrocarbon-enriched aromatic fraction and quinone-enriched polar fraction are more potent in inducing oxidative stress

Induction of oxidative stress by organic DEP chemicals
What is oxidative stress?

- Normal Redox equilibrium
- Oxygen radical production
- Oxygen radical inactivation

Oxidative Stress

↓ GSH  
↑ GSSG
Oxidative Stress Induces a Stratified Cellular Response

DEP Study Summary

Organic DEP chemicals induces adverse biological effects through the generation of stratified oxidative stress response

Which particle type in the ambient air is most dangerous and why?

...... PM$_{10}$ ?
...... PM$_{2.5}$ ?
...... Ultrafine particles
Transmission Electronmicrograph of a PM$_{2.5}$ Particle

PM$_{2.5}$ = aerodynamic diameter < 2.5 micron

Main sources are soil, crustal elements, sea salt etc.

May contain some emission particles

EPA regulated (mass standard)

(Courtesy Dr Sheldon Friedlander, UCLA)

Nel. Science, 308: 804-06, 2005
Main sources are emission particles or condensation of vapors

Diesel exhaust particles

Not EPA regulated (will require a numbers standard)

(Courtesy Dr Sheldon Friedlander, UCLA)
Characteristics of Harmful Inhaled Particles

1. Small size
2. Large surface area
3. Ability to be taken up
4. Toxic chemicals
Oxidant Potential and PAH Content of Different Size Particles

Li et al. Environmental Health Perspectives. 2003
The Cellular Effects of Different Size Particles

Control Coarse Fine UFP

Control UFP

HO-1 luciferase reporter transgenic mice

Filtered air PM$_{2.5}$ Ultrafine
Summary of Particle Size Comparison

Ultrafine particles are more dangerous than coarse and fine particles due to their ability to carry more pro-oxidative organic chemicals and stronger oxidant potential

How do ambient particles impact allergic airway inflammation such as asthma?
Experimental DEP Effects on Asthma

Allergen

PM → Allergen → Adjuvant

Sensitization

PM → Allergen → ↑ Airway inflammation

Allergic inflammation

PM → ↑ Airway Reactivity

Wheezing
DEP Augments Allergen-specific IgE in Humans


Graph showing the augmentation of allergen-specific IgE in humans with DEP exposure, with a significant increase observed on Day 4.
Animal Model to Study PM Effect on Allergic Sensitization

Allergic sensitization protocol

<table>
<thead>
<tr>
<th>Day</th>
<th>Intranasal</th>
<th>Inhalation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PM</td>
<td>1% OVA</td>
</tr>
<tr>
<td>2</td>
<td>PM + OVA</td>
<td>1% OVA</td>
</tr>
<tr>
<td>4</td>
<td>PM + OVA</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>PM + OVA</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>PM + OVA</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>Necropsy</td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Li et al, Environ Health Perspect, 2009
Ambient UFP enhanced OVA-induced allergic inflammation

**BAL Differential Cell Count**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Ctrl</th>
<th>OVA</th>
<th>UF#1</th>
<th>UF#1/OVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mono</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosino</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lympho</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutro</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**OVA-IgG1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Ctrl</th>
<th>OVA</th>
<th>UF#1</th>
<th>UF#1+OVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVA-IgG1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**OVA-IgE**

<table>
<thead>
<tr>
<th>Group</th>
<th>Ctrl</th>
<th>OVA</th>
<th>UF#1</th>
<th>UF#1+OVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVA-IgE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Li et al, Environ Health Perspect, 2009
Ambient Fine/UF particles failed to exert an adjuvant effect on OVA sensitization.
UFP-induced eosinophil infiltration was accompanied by increased IL-5 in BAL

Ambient UFP increased IgG1 and IgE production in OVA-sensitized mice
UFP-induced Allergic Inflammation in Nasal Maxilloburbinates

Nasal morphometry

Mucus

Eosinophils

Li et al, Environ Health Perspect, 2009
UFP-induced allergic inflammation in conducting airways

Li et al, Environ Health Perspect, 2009
Schematic drawing of the murine respiratory tract highlighting the intrapulmonary sites where pro-oxidative UFP exert its adjuvant effect on OVA sensitization.

Li et al, Environ Health Perspect, 2009
Morphometric Analysis of Mucosubstances and Eosinophils in Proximal and Distal Axial Airway

Proximal Axial Airway

Generation 5

Volume Density (nl/mm² basal lamina)

Saline  OVA/UF  OVA

Distal Axial Airway

Generation 11

Volume Density (nl/mm² basal lamina)

Saline  OVA/UF  OVA

Eosinophils/mm basal lamina

Saline  OVA/UF  OVA

Li et al, Environ Health Perspect, 2009
### Analyses of PM chemical composition and redox activity

#### PM Chemical Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>UF#1</th>
<th>F/UF#1</th>
<th>UF#2</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Total PAH Content

<table>
<thead>
<tr>
<th>PM Type</th>
<th>Total PAH Content (pg/µg PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF#1</td>
<td>240</td>
</tr>
<tr>
<td>F/UF#1</td>
<td>40</td>
</tr>
<tr>
<td>UF#2</td>
<td>160</td>
</tr>
</tbody>
</table>

#### DTT Consumption

<table>
<thead>
<tr>
<th>PM Type</th>
<th>DTT Consumption (nmol/µg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UF#1</td>
<td>0.05</td>
</tr>
<tr>
<td>F/UF#1</td>
<td>0.02</td>
</tr>
<tr>
<td>UF#2</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Li et al, Environ Health Perspect, 2009
Single intranasal administration of ng quantities of UFP is sufficient to enhance allergic sensitization to co-administered OVA.

500 ng of UFP

*p < 0.05 compared to Saline and OVA
Relevance of the Secondary IR:
Asthma flares in atopics after a sudden surge in ambient PM levels
PM Exposure in Mobile Laboratory near 110 Freeway

*MSU Mobile Lab in LA, CA

Interstate 110

VACES & Exposure Chambers in AirCARE 1
Ambient UFP enhance secondary allergic response in already-sensitized mice

**OVA-IgE**

- Saline
- OVA
- OVA/UFP

**Eosinophils**

- Saline
- OVA
- OVA/UFP

**OVA-IgG1**

- Saline
- OVA
- OVA/UFP

**2° Challenge:**
- OVA/FA
- OVA/UFP

* Ambient UFP enhance secondary allergic response in already-sensitized mice.*
UFP inhalation during the secondary immune response further increased cytokine gene expression in the lung.

**Eotaxin**

2° Challenge:
- OVA/FA
- OVA/UFP

**Muc5ac**

**IL-5**

**IL-13**

Fold Change Relative to Saline/OVA/FA
The target sites of ambient UFP during the primary immune response
The distal lung is the target of ambient UFP during the secondary immune response.
UFP Inhalation-enhanced allergic inflammation in the distal lung is accompanied by oxidative stress

**Sensitization**

- **OVA**
- **OVA + UFP**

**Challenge**

- **OVA/FA**
- **OVA/UFP**

**Ym1 (Chi3l3)**

<table>
<thead>
<tr>
<th>Sensitization</th>
<th>Fold Change Relative to Saline/OVA/FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1</td>
</tr>
<tr>
<td>OVA</td>
<td>5.0%</td>
</tr>
<tr>
<td>OVA/UFP</td>
<td>15.0%</td>
</tr>
</tbody>
</table>

**Organic compounds**
- Elemental carbon
- Nitrate
- Sulfate
- Total metals
- Unidentified

**Ammonium**

- 81.6%
- 16.6%
- 1.5%
- 1.0%
- 3.2%
- 6.8%
- 4.3%

**Elemental carbon**

- 6.8%
- 16.6%
- 1.5%
- 1.0%
- 3.2%
- 81.6%
- 4.3%
The target sites of ambient UFP during the secondary immune response

Intrapulmonary Sites of Secondary Immune Response:
- Proximal Axial Airway (G5)
- Distal Axial Airway (G11)
- Terminal Bronchiole
- Alveolar Duct (OVA/UFP/CAP only)
- Alveolus (OVA/UFP/CAP only)

Nasal Airway (OVA Sensitization Site)
- Larynx
- Trachea
- Left Lung Lobe
- Heart
- Right Lung Lobes

Centriacinus Magnified
Summary of Animal Studies

- Ambient UFP are capable of acting as an adjuvant to enhance immune response to experimental allergen
- UFP are able to interfere with both primary and secondary immune response
- There is a close correlation between the adjuvant effect of PM and particulate redox-active organic chemical content
- Real-life inhalation of pro-oxidative UFP during allergen challenge could lead to a profound allergic inflammation deep in the lung in already-sensitized animals
Induction of oxidative stress by particle-associated organic chemicals plays an important role in the adverse health effects of particulate pollutants.

UFP are more dangerous than coarse and fine particles.

UFP are capable of enhancing both primary and secondary immune response to an experimental allergen.

The adjuvant effect of PM is closely correlated to particles’ organic chemical content and oxidant potential.

Real-life inhalation of UFP during secondary allergen exposure could lead to a profound allergic inflammation deep in the lung in prior sensitized animals.

Relevance to human: The boosting effect of UFP inhalation on the secondary immune response may provide an explanation for the asthma flares after a sudden surge in ambient PM level.
Acknowledgements

**UCLA (Chemistry, toxicology, immunology)**
Dr. John Froines
Dr. Arthur Cho
Dr. Andre Nel
Dr. Arantza Eiguren
Debra Schmitz
Emma Di Stefano
Meiying Wang

**UC Irvine (Inhalation)**
Dr. Michael Kleinman
Dr. Dianne Meacher
Glenn Gookin

**MSU (Inhalation, pathology)**
Dr. Jack Harkema
Dr. Jim Wagner
Lori Bramble
Ryan Lewandowski

**USC (Environmental engineering)**
Dr. Constantinos Sioutas
Dr. Zhi Ning
Payam Pakbin

**UW-Madison (Chemistry)**
Dr. James Schauer