Innate-Type Allergy and Air Pollution

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New Perspectives: Addressing the Asthma and Allergy Epidemics, CURES Wayne State University, October 7, 2015
Outline for Talk

• Air pollution and allergic airway diseases (exacerbation and early onset)

• Innate- and adaptive-type allergy

• Ozone-induced type 2 airway immunity in mice

• Summary, future directions and questions
Air Pollution and Allergic Airway Disease

- Air pollution causes 3.3 million deaths/year worldwide
- 2.5 million disability-adjusted life years were attributable to ambient ozone (O3) exposure alone in 2010
- Air pollutant exposure exacerbates pre-existing allergic rhinitis and asthma
- Ozone and airway allergy are predicted to increase with climate change
- Do air pollutants contribute to the onset of airway allergy?
Initiation and propagation of type 2 immune responses


Influx of eosinophils
Mucous cell metaplasia
Epithelial hyperplasia
Ym1/2 proteins
Airway Hyper-responsiveness
Our Overarching Hypothesis

Repeated exposures to ozone elicit innate-type allergy in the nose and lung.
Aim 1: To determine the onset of ozone-induced eosinophilic rhinitis and nasal type 2 immunity

- C57BL/6 male mice
- 0 or 0.5 ppm ozone (4h/day) for 1, 2, 4 or 9 weekdays
- Nasal histopathology
- Immunohistochemistry and morphometric analysis
- qRT-PCR for relative mRNA expression of selected inflammatory cytokines and airway epithelial proteins

Nasal epithelial thickness and granulocytes with increasing days of exposure

A

Nasal mucosa

1 d air (24 h)

1

Neutrophils

1 d ozone (2 h)

4

Eosinophils

1 d ozone (24 h)

7

9 d ozone (24 h)

10

B

Epithelial thickness (μm)

Days of exposure (h post-exposure)

Air

Ozone

1 (2 & 24)

2 (24)

4 (24)

9 (24)

C

Granulocyte influx % of mucosa

Days of exposure (h post-exposure)

Air

Ozone

1 (2 & 24)

2 (24)

4 (24)

9 (24)
Nasal epithelial protein and mRNA expressions with increasing days of exposure

A

Mucosubstances

YM1/YM2 proteins

1 d ozone (24 h)

9 d ozone (24 h)

B

Days of ozone exposure (h post-exposure)

1

2

4

9

1 (24)

2 (24)

4 (24)

9 (24)

Days of ozone exposure

C

Days of ozone exposure

Cica1

Il1b

Il13

Aim 2: To determine the role of lymphoid cells in ozone-induced eosinophilic rhinitis and nasal type 2 immunity

- Lymphoid cell-deficient Rag2(-/-)Il2rg (-/-) and Lymphoid cell-sufficient C57BL/6 mice
- 0 or 0.5 ppm ozone (4h/day) for 9 days
Aim 3: Determine the role of ILCs in ozone-induced eosinophilic rhinitis and nasal type 2 immunity

- Lymphoid cell-deficient Rag2(-/-), IL2rg(-/-), lymphoid cell-sufficient C57BL/6 mice, ILC-sufficient and T & B cell-deficient Rag2(-/-) mice
- 0 or 0.8 ppm ozone (4h/day) for 9 days
Aim 4: Determine if repeated exposure to ozone induces innate-type allergic airway responses in the lung

- Lymphoid cell-deficient Rag2(-/-)IL2rg (-/-), lymphoid cell-sufficient C57BL/6 mice, ILC-sufficient and T & B cell-deficient Rag2(-/-) mice
- 0 or 0.8 ppm ozone (4h/day) for 1 or 9 days
- Bronchoalveolar lavage fluid analysis for cells and cytokines
- Pulmonary histopathology
- Morphometric analysis
- qRT-PCR for mRNA expression of inflammatory cytokines and epithelial proteins
Ozone-induced eosinophils only in ILC-sufficient mice exposed for 9 days
Bronchiolar epithelial injury and cell proliferation after 1 day of ozone exposure in both ILC-sufficient and -deficient mice

BrdU-positive density /bronchial epithelial nuclei (%)

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<tr>
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<th>Air</th>
<th>Ozone</th>
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<td>C57BL/6</td>
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<tr>
<td>Rag2/-</td>
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<td>Rag2/- IIrg/-</td>
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Proximal Axial Airway

Distal Axial Airway

G5

G11
Mucous cell metaplasia in bronchiolar epithelium after 9 days of ozone exposure in ILC-sufficient mice, but not in ILC-deficient mice.

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Mucus- and type 2 immune-related mRNA overexpression in the lungs of ILC-sufficient mice after 9 days of ozone exposure
Repeated exposures to ozone elicit innate-type allergy in the nose and lung of mice, that is likely to be dependent on type 2 cytokine-producing innate lymphoid cells. This suggests a new paradigm for the epidemiologic association of air pollution and allergic airway diseases.
Future Aim:
Role of Alarmins

Kazuyoshi Kumagai, Chee Bing Ong, Daven Jackson-Humbles, Ryan Lewandowski, Nick Buglac, Phil Brook, Ning Li, and James Wagner

Questions?
Rationale for Mouse Exposures to Ozone

- Based on previous studies in ozone-exposed rodents and humans (Hatch et al. 2013, 1994), the respiratory dose/response to **0.8 ppm** ozone in mice is equivalent to **0.15 ppm in exercising humans** (people are 5x more sensitive to ozone than mice).

- Concentration of 0.15 ppm is approximately twice as much as that of the current U.S. 8 h NAAQS for ozone (**0.070 ppm**).

\[
\begin{align*}
0.8 \text{ ppm} & \quad \overset{5x}{\longleftrightarrow} \quad 0.15 \text{ ppm} \quad \overset{2.1x}{\longleftrightarrow} \quad 0.070 \text{ ppm} \\
\end{align*}
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Equivalent Dose/Response

National Ambient Air Quality Standards (NAAQS) for ozone
Recent findings by others: Single high ozone exposure, ILC2s, Eosinophilic Inflammation, Balb/c versus C57BL/6 mice

Recent findings by others:
Single high ozone exposure, ILC2s, AHR, Balb/c mice