Airborne Particle Effects on the Respiratory System of Sensitive Animals and Asthmatic Humans

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Rationale

- Studies suggest an association between ambient particulate matter and increased morbidity in individuals with allergic airway disease.

- Brown Norway rats can serve as a model of allergic airway disease due to a strong Th2 response following sensitization and challenge with OVA.
Study Objective One

To establish a model of airway reactivity in the Brown Norway rat through a process of sensitization and repeated challenge with ovalbumin.
Approach

- Characterize airway reactivity in BN rats sensitized and challenged with OVA
- Quantify intraepithelial cell mucosubstance content of the airways
- Measure changes in eosinophils and mast cells in the lungs
- Measure the degree of inflammation present in the lungs (centriacinar regions)
Activity Time-line

sensitization aerosolized ovalbumin airway challenges animals studied

DAY

10 13 16 19 21 24 27
EC200RL Assay: the effective concentration of methacholine required to double lung resistance
Effects of Ovalbumin Sensitization and Aerosol Challenge on Airway Hyperresponsiveness in Brown Norway Rats

* significantly different from control and sensitized/not challenged groups
### Histochemical Staining of Central Airway

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sensitized</th>
<th>Challenged</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&amp;E</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
</tr>
<tr>
<td>AB/PAS</td>
<td><img src="image4" alt="Image" /></td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
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<tr>
<td>CEM</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td><img src="image9" alt="Image" /></td>
</tr>
</tbody>
</table>
Left Lung Corrosion Cast
Epithelial Cell Volume of the Central Airway

Epithelial Volume

<table>
<thead>
<tr>
<th>Condition</th>
<th>Volume/Surface Area (BL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>~5 ± 1 µm²/µm³</td>
</tr>
<tr>
<td>sensitized</td>
<td>~8 ± 1 µm²/µm³</td>
</tr>
<tr>
<td>sens + chall</td>
<td>~14 ± 2 µm²/µm³</td>
</tr>
</tbody>
</table>

*Significant difference compared to control and sensitized conditions.
Alcian Blue/Periodic Acid Schiff Staining of Central Airway Epithelium

EPITHELIUM OF PROXIMAL AIRWAY
Control Animal H&E Stain

Challenged Animal H&E Stain
Volume of Intracellular Mucosubstances of the Central Airway

Mucosubstance Volume

Volume/Surface Area (BL) (µm³/mm²)

- control
- sensitized
- sens + chall

* Significant difference
Perivascular (PV) Mast Cells and Eosinophils
Number of PV Mast Cells

Mast Cells

control sensitized sens + chall

Number/Basal Length (mm)
Number of PV Eosinophils

Eosinophils

Number/BL Length (mm)

control  sensitized  sens + chall
Study Objective Two

- To determine the most optimal conditions for a model of allergic airways in the Brown Norway rat by repeated weekly challenges with ovalbumin.
Activity Time-line

- Sensitization
- Aerosolized ovalbumin airway challenges
- Animals studied

DAYS: 14, 21, 28, 35
EC200RL: The effective dose required to double lung resistance
Central Airway Wall Composition
# Volume of Mucin Present Within the Central Airways of Brown Norway Rats

The table below provides the volume of mucin (in um²) measured in different groups and weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>FA (--)</th>
<th>FA/S (*)</th>
<th>Challenged Wk 1</th>
<th>Challenged Wk 2</th>
<th>Challenged Wk 3</th>
<th>Challenged Wk 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>um² mucin</td>
<td>0.563</td>
<td>0.485</td>
<td>0.514</td>
<td>1.405</td>
<td>1.388</td>
<td>1.323</td>
</tr>
<tr>
<td>std err</td>
<td>0.176</td>
<td>0.162</td>
<td>0.203</td>
<td>0.41</td>
<td>0.145</td>
<td>0.268</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

*FA/S (*) indicates a significant difference compared to the FA (--) group.*
Effects of OVA on Eosinophils and Mast Cells

![Graph showing changes in Eosinophils and Mast Cells in the epithelium of the airway over weeks 1 to 4.](image-url)
Eosinophil Increases Following OVA Challenges

Eosinophils and Mast Cells in the Submucosa of the Airway

Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td></td>
</tr>
<tr>
<td>FA/S</td>
<td></td>
</tr>
<tr>
<td>Challenged Week 1</td>
<td></td>
</tr>
<tr>
<td>Challenged Week 2</td>
<td></td>
</tr>
<tr>
<td>Challenged Week 3</td>
<td></td>
</tr>
<tr>
<td>Challenged Week 4</td>
<td></td>
</tr>
</tbody>
</table>

[Bar chart showing eosinophil and mast cell percentages across different groups and weeks.]
Blood Vessel Scoring of Perivascular Cell Influx

None (0)  
Mild (+)  
Moderate (+++)  
Severe (++++)
High Levels of Inflammation in Perivascular Space

Percentage of Sites with Inflammation

Perivascular Space
Percentage of Sites with Inflammation

Percentage of Sites Involved

FA  FA/S  Challenged Week 1  Challenged Week 2  Challenged Week 3  Challenged Week 4

Groups
Cellularity in the Perivascular Space

Groups:
- FA
- FA/S
- Challenged Week 1
- Challenged Week 2
- Challenged Week 3
- Challenged Week 4

Percent
Mast Cells

Mast Cells in the Perivascular Space

- FA
- FA/S
- Challenged Week 1
- Challenged Week 2
- Challenged Week 3
- Challenged Week 4

Percent
Eosinophils

Eosinophils in the Perivascular Space

Percent

Groups

FA
FA/S
Challenged Week 1
Challenged Week 2
Challenged Week 3
Challenged Week 4
Transverse Lung Tissue Sections
Centriacinar Region (BADJ) Scoring
Centriacinar Regions

Centriacinar Regions
Percentage of Sites with Inflammation

Groups
- FA
- FA/S
- Challenged Week 1
- Challenged Week 2
- Challenged Week 3
- Challenged Week 4

Percentage of Sites Involved

p<0.05 compared with FA control
p<0.05 compared with FA/S control
Study Objective Three

- To determine if exposure to PM will alter airway reactivity, lung inflammation, the immune response and epithelial injury in Brown Norway rats sensitized and challenged with OVA.
Hypotheses

- OVA-specific serum IgE and eosinophilic inflammation will be increased in an allergic model and may be changed by PM exposure immediately following allergen challenge.

- Detection of a PM effect on airway reactivity and cell permeability may be enhanced in a Brown Norway rat model of allergic airway disease.

- Ammonium nitrate and carbon are the two most prevalent forms of PM in California.
Exposure

Day 0: Sensitization (OVA subQ)

Day 14: Challenge (OVA aerosol)

Exposure: (6 hr/day x 2 days)

PM

FA
Assays

- Serum IgE: OVA-specific
- PFT: Airway responsiveness (MC)
- BAL: Protein, total cell number, and differential
- Cell permeability: Ethidium homodimer-1
- Cell proliferation: BrdU
PM Composition

- Ammonium nitrate  150 µg/m³
- Carbon black  100 µg/m³
- Particle size (MMAD)  1.0-1.5 µm
Photomicrograph of Collected Ovalbumin Particles
Photomicrograph of Collected PM Particles
PM Samples for Carbon Analysis
Ion Chromatograms of PM Particle Samples during exposure PM 41
OVA-Specific Serum IgE

% Positive Control

N/C  S  S/C

FA  PM
Pulmonary Airway Responsiveness

![Graph showing EC 200 RL for FA and PM.](image-url)
Protein in BAL

![Bar chart showing protein levels in BAL](image)
Eosinophils in BAL

- FA
- PM

Percent

S
S/C
Epithelial Cell Permeability
Cell Permeability

![Graph showing relative scoring for E+D-1 cell staining]

- **Relative Scoring for E+D-1**
- **Cell Staining**

- **S**:
  - FA
  - PM

- **S/C**:
  - FA
  - PM
Mucin Volume of Airway Epithelium
Epithelial Cell DNA Synthesis

FILTERED AIR

PARTICULATE MATTER

100 μm

20 μm
Airway Epithelial Labeling

Percent BrdU Positive Cells

S

S/C

FA
PM

*
Histological Analysis, Eosinophils
Exposure

Day 0
Sensitization (OVA subQ)
Day 14
Challenge (OVA aerosol)

Days 15-17
Exposure (6 hr/day x 3 days) FA & PM

Days 18-19
Non-Exposure (2 days)

Days 20-22
Exposure (6 hr/day x 3 days) FA & PM

Days 23-24
Non-Exposure (2 days)
mRNA Levels in Lung Tissues of BN Rats Exposed to FA or PM

OVA-Sensitized/Challenged Brown Norway Rats

![Graph showing mRNA levels in lung tissues of BN rats exposed to FA or PM](image-url)
Study Objective Four

- To determine the effects of exposure to PM in human asthmatic volunteers on airway biopsy tissue gene expression for a panel of cytokines.
Tissue: IL-12p35 mRNA

Data: mean ± SE

↓ Ct = ↑ expression
In-Vivo Control Allergen PMA

PCR Cycles

Tissue: IL-15 mRNA

Data: mean ± SE

↓ Ct = ↑ expression
In-Vivo Control Allergen PMA

Tissue: IL-10 mRNA

Data: mean ± SE

↓ Ct = ↑ expression

PCR Cycles

In-Vivo  Control  Allergen  PMA
Conclusions

- Brown Norway rats can serve as a reasonable model of allergic airway disease following a single sensitization and challenge with OVA.
- PM may serve as an adjuvant and increase systemic levels of IgE following sensitization and challenge in Brown Norway rats.
- Airway hypersensitivity was not changed by sensitization and challenge in this model. Exposure to carbon and ammonium nitrate PM may actually decrease response to MC challenge.
Conclusions

- Airway epithelial cell permeability to ethidium homodimer-1 in our model may increase following two days of exposure to carbon and ammonium nitrate particles (N.S.)

- Significant increases in airway epithelial cell BrdU labeling occur in this model of allergic airway disease following exposure to carbon and ammonium nitrate PM (p<0.05)

- Centriacinar alveolitis may be increased by PM exposure in a Brown Norway rat model of allergic airway disease (N.S.)
Conclusions

- Human airway biopsies can be used to examine gene expression for the variety of different cytokines.

- Gene expression of cytokines within human airways is not significantly altered following acute exposure to carbon and ammonium nitrate PM.

- IL-5 gene expression in Brown Norway rats is transiently altered following acute exposure to carbon and ammonium nitrate PM.

- Combined animal and humans studies can be used to provide new insights on potential mechanisms of the health effects of PM on the respiratory system.
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- William Walby
- Valerie Mitchell
- Maria Suffia
- Julian Recendez
- Andrew Goodyear
- Marzieh Shafii
Exposure

Sensitization (OVA subQ)

Challenge (OVA aerosol)

Exposure (6 hr/day x 3 days) FA & PM

Non-Exposure (2 days)

Exposure (6 hr/day x 3 days) FA & PM

Non-Exposure (2 days)

DAYS

0 14 15-17 18-19 20-22 23-24