INTERACTION OF AN ASTHMA PROMOTING IL4RA ALLELE WITH OXIDATIVE STRESS PATHWAYS

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Overall objective

- To study the role gene-environment interactions in promoting the development of asthma
Asthma

- 20 million
- 10 million allergic asthma
- Increase in the prevalence 75% from 1980-1994
- Children < 5 asthma rates increased >160% from 1980-1994
Asthma

Environmental Factors

Genetic polymorphism

Inter-individual variability
Air Pollution

- Ozone (\(O_3\))
- Particles
- Sulfur Dioxide (\(SO_2\)) sulfur
- Oxides of Nitrogen (\(NO_x\))
- Volatile Organic Compounds (VOCs)
The impact of particulate pollutants on asthma

- Cardiorespiratory morbidity and mortality
- Asthma flares
  1. Increased symptom score
  2. Requirement for more frequent medication
  3. Hospitalization
Particulate pollutants & allergic sensitization

- Children who live near motorways have increased incidence asthma
- In humans intranasal co-administration of Diesel exhaust particles (DEP) and neo-antigen (KHL) → primary sensitization and anti-KHL specific IgE in 9 of 15 atopic patients

J Allergy Clin Immunol 1999;1183-8
Murine Models

Particle exposure during antigen sensitization increases:

- Airway hyper-reactivity
- Airway inflammatory cells
- Number of goblet cells
- Antigen specific IgE levels
- Increase in pro-allergic T helper 2 cytokine profile (Th2) IL-5, IL4, IL-13
- Decrease in T helper 1 cytokine IFN-g
Particles

- Coarse 2.5–10 µm
- Fine ≤2.5 µm
- Ultrafine ≤0.1 µm
- Diesel Exhaust Particles (DEP) (composed of fine and ultrafine particles)
Particle Composition

*Organic Carbons: polycyclic aromatic hydrocarbons (PAH) and quinones

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>Claremont (n = 3)</th>
<th>USC (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coarse</td>
<td>Fine</td>
</tr>
<tr>
<td>Mass concentration (µg/m³)</td>
<td>12.3</td>
<td>17.3</td>
</tr>
<tr>
<td>Organic carbon (%)</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>Elemental carbon (%)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Nitrate (%)</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>Sulfate (%)</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Metals/total elements (%)</td>
<td>51</td>
<td>13</td>
</tr>
</tbody>
</table>

Values represent the mean fractional composition (%) in which SEM varied < 10%.

Role of oxidative stress in the health effects of particulate pollutants

- Oxidative stress is a state of redox disequilibrium
- Decrease in the cellular glutathione (GSH)/glutathione disulfide (GSSG) ratio
- Activates a number of the redox-sensitive signaling cascades
- Responses that could be protective or injurious in nature
Oxidative stress promotes dendritic cell pro-allergic Th2 skewing

- DEP induced oxidative stress inhibits TH1 immunity in response to TLR agonist
- TH1 immunity restored with administration of antioxidant, N-acetylcysteine

Asthma

Environmental Factors

Genetic polymorphism

Inter-individual variability
IL4Rα polymorphism, Q576R

- Severe asthma
- Severe RSV bronchiolitis
- Rapid decline in lung function in smokers
- Heightened allergen sensitization in the context of maternal smoking
- 70% allele frequency in African Americans vs. 20% in Caucasians, 50% and 4% homozygosity, respectively
Q576R mutation promotes intense allergen-induced airway inflammation and remodeling

- Increased peribronchial and perivascular inflammation
- Increased goblet cell
- Increased bronchoalveolar lavage (BAL) fluid eosinophils
- Sub-epithelial cell fibrosis
- Augments IL-4R–dependent signaling

Specific Aim

- Study the impact of Q576R X Diesel exhaust particles (DEP) interaction on allergen induced airway disease.
- Hypothesis
  - DEP acts as an adjuvant to promote allergic airway sensitization
  - Q576R synergizes with DEP exposure to promote heightened allergic airway inflammation
In-vivo study design:

6-8 week old

WT

Q576R

Intranasal Sensitization
1. Saline
2. UFP
3. OVA
4. UFP+OVA

3 day 1% OVA aerosol challenge
Study Design

WT

Total IgE & OVA-IgE ELISA

Q576R

Bronchoalveolar lavage (BAL):
Total cell # & diff IL-4, IL-13, IL-6 IL-17A, INF-γ

Lung histochemical analysis, PAS staining
In-vitro studies

- DEP acts as an adjuvant to promote allergic airway sensitization
Mechanism of DEP associated allergic sensitization

- Oxidative stress
- Prelim data: Chatila lab performed gene microarray of DEP exposed human dendritic cells
- Increase in genes in the oxidative stress pathway
- Increase in Jagged1
Notch Th1 vs. Th2

Notch pathway and asthma

- May program cells toward proallergic Th2 vs Th1 pathways
- Jagged 1 or Jagged 2 + Notch 1 or 2 → Th2
- DLL1 or DLL4 + Notch3 → Th1
- Administration of Notch pathway inhibitor, Gamma Secretase Inhibitor (GSI) inhibits asthma features

Am J Respir Crit Care Med. 2009 May 15;179(10):875-82
In-vitro protocol

Murine bone-marrow

DEP X 24 hr: 2.5ug cm$^2$ - 15ug cm$^2$

Flow-cytometry
DC confirmation & protein expression

Quantitative PCR
gene expression
DC culture led to 60-70% DC purity

Dendritic cell Gate

Unstained sample

CD11c+
DC culture treatment with DEP results in up to 20X increase in Jagged 1 expression

(n=3, paired t-test, p=0.0056, 99% CI -27.73 to -4.00)
DC culture treatment with DEP results in 40% decrease in Notch 1 expression.

(n=3, paired t-test, p = 0.04, 95% CI 0.025 to 0.712)
DC culture treatment with DEP results in a reduction of DLL1

![Graph showing the reduction of DLL1 with DEP treatment.](image)

Legend:
- **Purple**: DLL1

Units ug/cm², n=3 per group

Treatment Group (n=3, Mann-Whitney test, p = 0.1)
No difference in Jagged 2 or Notch 2 gene expression
No difference in Notch 3 or Notch 4 gene expression
No difference in DLL4 or DLL3 gene expression
Flow results

Unstained Control

CD11c+ Gate
DEP treatment results in suppression of DLL1

Gate CD11c+

Red: DEP treated
Blue: Untreated
Purple: Negative Control

n=3
DEP treated group decreased
Notch 2

Gate CD11c+

Red: DEP treated
Blue: Untreated
Purple: Negative Control

n=3
No difference in Notch 3 and DLL4
Ongoing experiments

DEP txd + DO11 T cells

Tx OVA

Proliferation, CFSE

PCR: IL-2, IL-4, IL-13, IFN-gamma, GATA-3, T-bet
Summary

- Preliminary results suggest that a potential mechanism by which DEP promotes allergic sensitization TH2 DC programming via the Notch pathway
- Assess for differences in the Q576R mice
- Confirm these results in-vivo
References


References


References


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