Pathogenesis of Occupational &
Environmental Asthma: new targets for
investigation
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Asthma and Lung Biology

Disclosures
• Research: GlaxoSmithKline, MedImmune, US EPA,
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Outline
• Review of immunopathogenesis of occupational asthma
• Innate Immune mechanisms involved in environmental
and occupational asthma
• Oxidative stress and environmental and occupational
asthma
Sensitization to occupational allergens

HMW vs. LMW sensitizers

Reference:

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High Molecular Weight Allergens

- Immunopathology typically due to IgE production to high molecular weight allergens
- Airway inflammation is typically eosinophilic
- Occupational sensitization to a given agent may be due to different allergens compared to other allergy associated with the same agent
  - Bakers asthma associated exposure to agents such as thioredoxin in wheat
  - Oral allergy due to wheat associated with omega 5 gliadin

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High molecular weight allergens

<table>
<thead>
<tr>
<th>Name</th>
<th>Clinical Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>Bakers asthma</td>
</tr>
<tr>
<td>Oat</td>
<td></td>
</tr>
<tr>
<td>Rye</td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td></td>
</tr>
<tr>
<td>Soy</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Peanut</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Tree nuts</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Corn</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Milk</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Shrimp</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Crab</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Fish</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Shellfish</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Insect Venom</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Egg</td>
<td>Oral allergy, asthma</td>
</tr>
<tr>
<td>Peppers</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Tomato</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Rice</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Mustard Seeds</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Sesame Seeds</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Sunflower Seeds</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Mustard Greens</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Sesame Greens</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Sunflower Greens</td>
<td>Oral allergy</td>
</tr>
<tr>
<td>Green Beans</td>
<td>Oral allergy</td>
</tr>
</tbody>
</table>

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Low molecular weight sensitizers

- Some cause disease via IgE mediated mechanisms
  - Phthalic anhydride
  - TMA
  - Chromium salts
  - Nickel salts
  - Epoxy amines
  - Penicillin

- Others involve mechanisms which are incompletely understood
  - Innate mechanisms
  - Cell mediated mechanisms
  - Isocyanates may have multiple mechanisms

Low molecular weight allergens

- Often linked to a single initial event
- Often not IgE mediated
- Likely involves epithelial cell injury
- Increased methacholine reactivity
- Traditionally called RADs
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New areas of investigation for OA

• Can we extrapolate lessons from the “hygiene hypothesis”?
• Can we extrapolate lessons from the biology of environmental/air pollution research to the unique exposures of OA?
• Specific interest in oxidative stress?

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Innate Immunity and OA

Animal handlers

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Pollutants shown to enhance recall response to allergen

- Ozone
- NO₂/SO₂
- Endotoxin
- Diesel Exhaust
- ETS

Responses include:
- IgE
- PMNs
- Eosinophils
- Airways Reactivity
Airborne Endotoxin Predicts Symptoms in Non–Mouse-sensitized Technicians and Research Scientists Exposed to Laboratory Mice


- Mouse sensitized workers had increased symptoms linked to allergen levels
- Non-mouse sensitized workers had increased symptoms associated with endotoxin levels
- Risk factors for both symptomatic groups included atopy and allergy to domestic pets
- Findings suggest interactions between LPS and allergic inflammation

Gene-environment interactions influence airways function in laboratory animal workers

Pacheco et al, JACI Vol 126(2), Pages 232-240, 2010

- Decreased FEV1 and FEF25-75 seen in endotoxin exposed workers with the CD14-1619AG/GG genotype
- Findings suggest a role for CD14/LPS biology in occupational lung disease

Logistic regression plots for the association between endotoxin exposure and current wheeze, stratified by CD14/-260 genotype.

Each symbol represents a group of workers with the same estimated exposure level. Associations were adjusted for sex, age, smoking habits and farm childhood.
Hexamethylene diisocyanate asthma is associated with genetic polymorphisms of CD14, IL-13, and IL-4 receptor α.


Interim summary: innate immunity

- Innate immunity results in:
  - Increased antigen presentation capability
  - Increased inflammation

- Innate immunity (CD14) influences symptoms and risk of disease
  - Bioaerosols
  - Event TDI

- Gene X exposure
  - CD14 TT genotype increased wheeze at low LPS
  - Decreased wheeze at high LPS
  - CD14 CC or CT associated with wheeze with high LPS

Oxidant Biology and Inflammation

- Multiple environmental pollutants are oxidant or pro-oxidant molecules (Romieu et al 2008)
- Native oxidant generation plays an important role in immune response (Gaudie et al, 2006)
GSTM1 and related genes

NRF2 nuclear transcription factor

- Epidemiological literature too extensive to review here. In summary, GSTM1 null individuals have increased risk for:
  - Ozone induced asthma exacerbation
  - PM induced lung and CV disease
  - Perinatal exposure to tobacco smoke and increased risk of asthma/wheezing disease
  - Associated with increased risk for isocyanate asthma and response to LPD
- GSTM1 Null challenge studies:
  - Associated with increased response to diesel exhaust
  - Associated with increased response to tobacco smoke

GSTM1 genotype and ozone response

Lung Function
Inflammation

Polymorphism of Quinone-metabolizing Enzymes and Susceptibility to Ozone-induced Acute Effects

GSTM1 genotype and ozone induced decreases in FEF25-75 in asthmatics

- GSTM1 null asthmatics have increased asthma symptoms with ozone exposure than do GSTM1 sufficient persons

**Table**

<table>
<thead>
<tr>
<th>Group</th>
<th>Male</th>
<th>Female</th>
<th>Ozone Exposure</th>
<th>FEF25-75</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1 null</td>
<td>20</td>
<td>10</td>
<td>Yes</td>
<td>0.45</td>
<td>0.34</td>
</tr>
<tr>
<td>GSTM1 sufficient</td>
<td>20</td>
<td>10</td>
<td>Yes</td>
<td>0.25</td>
<td>0.20</td>
</tr>
</tbody>
</table>

**Graph**

Alexis et al, JACI 2009

The glutathione-S-transferase Mu 1 null genotype modulates ozone-induced airway inflammation in human subjects.

Alexis, NE et al, Journal of Allergy and Clinical Immunology 124(6):1222-8; 2009

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GSTM1 genotype and PM response

Lung Function

Inflammation
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**DEP effect on the response to allergen stratified by GSTM1 genotype**


<table>
<thead>
<tr>
<th>Response</th>
<th>Clean air and allergen</th>
<th>DEP and allergen</th>
<th>Difference</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE (U/mL)</td>
<td>9·8 (6·4)</td>
<td>121·2 (34·1)</td>
<td>111·4 (29·7)</td>
<td>&lt;0·002</td>
</tr>
<tr>
<td>Interleukin 4 (U/mL)</td>
<td>0·2 (0·1)</td>
<td>6·0 (5·0)</td>
<td>5·7 (4·0)</td>
<td>&lt;0·0001</td>
</tr>
<tr>
<td>Interferon γ (ng/L)</td>
<td>1·2 (0·6)</td>
<td>0·0 (0·0)</td>
<td>-0·2 (0·0)</td>
<td>&lt;0·002</td>
</tr>
<tr>
<td>Interferon γ/Interleukin 4</td>
<td>4·6 (2·7)</td>
<td>0·4 (1·4)</td>
<td>0·1 (0·3)</td>
<td>&lt;0·0001</td>
</tr>
<tr>
<td>Histamine (nmol/L)</td>
<td>3·1 (1·3)</td>
<td>15·0 (7·4)</td>
<td>12·9 (7·0)</td>
<td>&lt;0·0001</td>
</tr>
</tbody>
</table>

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**DEP effect on the response to allergen stratified by GSTM1 genotype**


<table>
<thead>
<tr>
<th>Median (interquartile range)</th>
<th>Clean Air + Allergen</th>
<th>SHS + Allergen</th>
<th>Difference</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE, Unit</td>
<td>12.2 (1.1–27.3)</td>
<td>101.5 (23.0–746.6)</td>
<td>89.3 (39–975.5)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>IL-4, Unit</td>
<td>3.2 (2.0–7.0)</td>
<td>3.5 (3.1–13.7)</td>
<td>0.3 (4.0–13.3)</td>
<td>0.0001</td>
</tr>
<tr>
<td>IFN-γ, ng/L</td>
<td>0.6 (0.1–1.6)</td>
<td>0.3 (0.1–1.4)</td>
<td>-0.3 (0.0–3.1)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Histamine</td>
<td>3.6 (2.0–6.8)</td>
<td>12.5 (3.0–24.7)</td>
<td>9.1 (3.0–20.6)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

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**NASAL RESPONSES IN 19 SUBJECTS AFTER EXPOSURE TO CLEAN AIR PLUS ALLERGEN OR SECONDHAND SMOKE EXPOSURE PLUS ALLERGEN, AND THE DIFFERENCES IN RESPONSE**


<table>
<thead>
<tr>
<th>Median (interquartile range)</th>
<th>Clean Air + Allergen</th>
<th>SHS + Allergen</th>
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<td>0.3 (4.0–13.3)</td>
<td>0.0001</td>
</tr>
<tr>
<td>IFN-γ, ng/L</td>
<td>0.6 (0.1–1.6)</td>
<td>0.3 (0.1–1.4)</td>
<td>-0.3 (0.0–3.1)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Histamine</td>
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</tr>
</tbody>
</table>
### Table 4

<table>
<thead>
<tr>
<th>Genotyping</th>
<th>n</th>
<th>IgE (U/ml)</th>
<th>IL-4 (U/ml)</th>
<th>IFN-γ (ng/L)</th>
<th>Histamine (nM)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1 Present</td>
<td>5</td>
<td>46.7 (9.2-65.0)</td>
<td>3.2 (0.0-5.4)</td>
<td>-0.5 (0.2-1.5)</td>
<td>-0.3 (-0.8-1.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>Null</td>
<td>14</td>
<td>4.0 (1.2-72.5)</td>
<td>4.0 (0.4-1.1)</td>
<td>1.5 (0.0-6.0)</td>
<td>0.3 (0.1-0.6)</td>
<td>0.33</td>
</tr>
<tr>
<td>GSTP1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile/Ile</td>
<td>13</td>
<td>2.9 (0.6-13.0)</td>
<td>3.3 (0.4-12.2)</td>
<td>-0.5 (0.0-0.5)</td>
<td>4.6 (0.1-10.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Ile/Val</td>
<td>6</td>
<td>5.1 (0.6-42.3)</td>
<td>3.3 (0.4-12.5)</td>
<td>-1.5 (0.0-6.0)</td>
<td>4.6 (0.1-10.5)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*p Value*

**Effect of GSTs genotypes on SHS/allergen response**


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**GSTM1 genotype and occupational asthma**

TDI

LPS

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### Slide 30

Table 4

<table>
<thead>
<tr>
<th>Genotyping</th>
<th>n</th>
<th>TDI (U/ml)</th>
<th>TDI (U/ml)</th>
<th>TDI (U/ml)</th>
<th>TDI (U/ml)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
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<td>5</td>
<td>46.7 (9.2-65.0)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>4.6 (0.1-10.5)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*p Value*
**Table 3**

N-Acetyltransferase genotypes as modifiers of diisocyanate exposure-associated asthma risk.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CI, 95%</th>
<th>HDI, 95%</th>
<th>MDI, 95%</th>
<th>TDI, 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1−</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Wikman, Harriet; Piirila, Paivi; Rosenberg, Christina; Luukkonen, Ritva; Kaaria, Katja; Nordman, Henrik; Norppa, Hannu; Vainio, Harri; Hirvonen, Ari.* Pharmacogenetics.  12(3):227-233, April 2002.

- Only statistically significant associations are shown.
- P for interaction 0.040.
- Crude OR.
- OR adjusted for age, gender, smoking and atopy.
- P for interaction 0.250.
- P for interaction 0.660.

**Baseline versus post-CCRE (endotoxin) responses (mean±SEM) of (A) circulating white blood cells (WBCs), (B) polymorphonuclear neutrophils (PMNs), (C) % PMNs in sputum and (D) sputum PMNs/mg sputum in GSTM1 sufficient and GSTM1 null volunteers.**

**Summary GSTM1**

- Oxidative stress likely plays a very important role in occupational asthma.
- GSTM1 and related genes modulate both acute response to occupational agents and risk for permanent disease.
- Potential development of biomarkers for exposure and risk of disease.
- Potential interventions.