Barnyard Dust Composition and Implications for Asthma in Children

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Washington, DC
• **Objective:** Identify components of barnyard dust extracts that mediate asthma protection *in vivo*

• **Rationale:** Asthma prevalence in children exposed to farming early in life is reduced over 5-fold compared to non-farming children, Amish dust extracts have been shown to enable Asthma protection in *in vivo* models.
Asthma affects approximately 300 million people worldwide and is the most prevalent chronic disease of childhood.
Asthma

Asthma causes

*The exact cause of asthma is not known. It is believed to be caused by a variety of factors.*
• Important role of the environment;

• Humans are exposed to a vast numbers of chemicals
Barnyard Dust Offers a Clue to Stopping Asthma in Children

By GINA KOLATA   AUG. 3, 2016
Scientists say they may have found a sort of magic ingredient to prevent asthma in children: microbes from farm animals, carried into the home in dust.

The results of their research, published on Wednesday in The New England Journal of Medicine, were so convincing that they raised the possibility of developing a spray to do the same thing for children who do not have regular contact with cows and horses.

Asthma and the Environment

The Opinion Pages

Health Secrets of the Amish
Moises Velasquez-Manoff   AUG. 3, 2016

In recent decades, the prevalence of asthma and allergies has increased between two- and threefold in the United States. These days, one in 12 kids has asthma. More are allergic.
Environmental monitoring with an *in vitro* bioassay

- **in vitro bioassay**
  - An air liquid interface (ALI) culture model of immortalized epithelial cells;
  - IL-6 endpoint;

- **Induction**: 5-7 days
- **Expansion**: 2-4 days
- **Maintenance**: 21+ days
Sample Extraction

**Polarity**

- Water
- Methanol
- Chloroform
- Dichloromethane
- Hexane

Barn Dust Extract

More Polar

Less Polar

More Polar

Less Polar
Sample Stability

Temperature and Pressure

Barn Dust

- 25 °C, 1 atm
- 80 °C, 103 atm
- 121 °C, 2 atm autoclave

✔ ✔ ✔
Sample Preparation

- Amish Barn Dust
- Aqueous extract
- Sterile Filtration (0.2 μm)
- Size Exclusion
  - 64-28 kDa
  - 51.5-42 kDa
- Enzymatic digestion
- Reversed Phase Separation
- Endotoxin Removal
SEC fractions and biological response
Sample Preparation

Affinity Separation - Lectins

SEC (42-51.5 kDa)

Affinity Chromatography

WGA

Ligand Motifs:
Sialic Acid
N-acetyl-D-glucosamine

ConA

Ligand Motifs:
α-D-mannosyl residues
α-D-glucosyl residues

Fraction Preparation:

1. Pipette 200 μl of resin slurry to spin column. Centrifuge column to remove storage buffer.
2. Wash resin 3X with 200 μl of Binding/Wash Buffer.
3. Dilute glycoprotein sample in Binding/Wash Buffer (4:1).
4. Add diluted glycoprotein sample to column and mix for 10 minutes.
5. Centrifuge and discard flow-through.
6. Wash resin with 400 μl of Binding/Wash Buffer 4X.
7. Add 200 μl of Elution Buffer and mix for 5-10 minutes.
8. Centrifuge. Collect eluate and repeat steps 7 and 8.
Affinity Separation - Lectins

Lectins:
Carbohydrate binding proteins

Targets:
- Glycoproteins
- Glycopeptides
- Peptidoglycans
- Glycolipids
- Glycosides
- Lipopolysaccharides
Affinity Separation - Lectins

Lectins-Glycoconjugate Interactions

<table>
<thead>
<tr>
<th>Group of bio-molecules</th>
<th>Range of affinity (Kd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lectin-oligosaccharide (Lectin-Glycoconjugate with multiple glycosylation)</td>
<td>$10^{-7} - 10^{-3}$ M ($&lt;10^{-7}$ M)</td>
</tr>
<tr>
<td>Complex Salt</td>
<td>$10^{-8} - 10^{-2}$ M</td>
</tr>
<tr>
<td>Protein A-IgG</td>
<td>$10^{-6}$ M</td>
</tr>
<tr>
<td>Antigen-antibody (monoclonal)</td>
<td>$10^{-9} - 10^{-7}$ M</td>
</tr>
<tr>
<td>Hormone-receptor</td>
<td>$10^{-12} - 10^{-5}$ M</td>
</tr>
<tr>
<td>Avidin-biotin</td>
<td>$10^{-15}$ M</td>
</tr>
<tr>
<td>Covalent bond</td>
<td>$10^{-60}$ M</td>
</tr>
</tbody>
</table>
## Total Organic Carbon (TOC) of fractions

<table>
<thead>
<tr>
<th>Sample</th>
<th>TOC (µg/mL)</th>
<th>% Initial TOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amish Dust Extract - &quot;B&quot;</td>
<td>6454</td>
<td>100</td>
</tr>
<tr>
<td>Dialyzed Amish Dust Extract</td>
<td>622</td>
<td>9.64</td>
</tr>
<tr>
<td>SEC 64-28 kDa - “DA-DD”</td>
<td>140</td>
<td>2.17</td>
</tr>
<tr>
<td>SEC 42-51.5 kDa - &quot;DB&quot;</td>
<td>33</td>
<td>0.52</td>
</tr>
<tr>
<td>WGA Retentate of DB - &quot;KO&quot;</td>
<td>5</td>
<td>0.08</td>
</tr>
</tbody>
</table>

![Deconvolution Progress Diagram](image-url)
Estimation of active components concentration

Based on KO TOC concentration:

Active Components in Raw dust:
~0.1 mg/g Dust

Active Components Concentration (Aqueous extract)*:
~0.2 µM
*Based on average MW of DB fraction

Dust Equivalent (DEQ) 100 mg/mL
1 mg DEQ dose = ~ 0.1 µg
Total Protein and Endotoxin Concentration on SEC fractions
Glycan Profiling

Lectin Array Principle

- Rapid and high-sensitivity profiling of complex glycan features without the need for liberation of glycans.
Glycan Profiles

Normalized Intensity to Positive Control

[Images of bar charts and dot plots showing glycan profiles for different conditions: B, DB, DC, KO]
Glycan Profiles

Top 7 interactions and specificities

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Carbohydrate specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con A</td>
<td>αMan, αGlc</td>
</tr>
<tr>
<td>DSA</td>
<td>(GlcNAc)2-4</td>
</tr>
<tr>
<td>Jacalin</td>
<td>Galβ3GalNAc</td>
</tr>
<tr>
<td>LBA</td>
<td>GalNAc(1,3)[αFuc(1,2)Gal</td>
</tr>
<tr>
<td>PHA-P</td>
<td>Galβ4GlcNAcβ2Manα6(GlcNAcβ4)</td>
</tr>
<tr>
<td>UDA</td>
<td>GlcNAc</td>
</tr>
<tr>
<td>VVA</td>
<td>GalNAc</td>
</tr>
</tbody>
</table>
## LC-QTOF Conditions

**Agilent 6540 Ultra High Definition (UHD) Accurate-Mass Quadrupole Time-of-Flight (Q-TOF)**

- Gas Temperature 300 °C
- Drying gas flow 13 L/min
- Nebulizer pressure 45 psig
- Sheath gas Temperature 400 °C
- Sheath gas flow 12 L/min
- Vcap 5000 V
- Fragmentor 175 V
- Nozzle Voltage 2000V
- Skimmer 65 V
- Column ZORBAX 300SB-C18 (2.1mm x 150 mm, 1.8 µm)
- Flow 0.5 mL/min
- Injection Volume 20 µL
- Column Temperature 60 °C
- Solvent A Water 0.1% Formic Acid
- Solvent B Acetonitrile 0.1% Formic Acid

<table>
<thead>
<tr>
<th>Time</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.00 min</td>
<td>95 %</td>
</tr>
<tr>
<td>2</td>
<td>16.00 min</td>
<td>0 %</td>
</tr>
<tr>
<td>3</td>
<td>18.00 min</td>
<td>0 %</td>
</tr>
<tr>
<td>4</td>
<td>18.01 min</td>
<td>95 %</td>
</tr>
<tr>
<td>5</td>
<td>20.00 min</td>
<td>95 %</td>
</tr>
</tbody>
</table>
LC-QTOF Data Analysis

Principal Components Analysis (PCA)

DB and DC pairs of treatments are (SEC fractions 1.5 min apart)
Principal Components Analysis (PCA)

3D PCA Plot

Entities with affinity to Lectins (WGA and ConA motifs)
LC-QTOF Data Analysis

Hierarchical Clustering of Entities and Sample Treatments

Entities present in B, DB, and Lectins retentates only
Hierarchical Clustering of Entities and Sample Treatments

Entities present in B, DB, and Lectins retentates only
Potential Target Entities

- 329 Entities common to B and DB
- 55 Entities common to B, DB and DB WGA Retentate