“In spite of the current plethora of industrial air pollutants, all such evils of mankind have not yet escaped from Pandora’s box…”

“As the Greek myth goes, curiosity opened Pandora’s box, and after all mankind’s evils had emanated, hope alone remained. A continuing spirit of investigative curiosity, it is hoped, will provide the answer to these questions.”

John Edmond Salvaggio, M.D. Quotes from his editorial in New England Journal Medicine 1970
Agriculture: Respiratory Illness in Large Animal Farming Environments

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## Disclosures

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<tr>
<td>Consultant/ Speakers bureaus</td>
<td>No Disclosures</td>
</tr>
<tr>
<td>Research funding</td>
<td>National Institute of Health: NIEHS, NIAID; National Institute of Occupational Safety and Health</td>
</tr>
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<td>Stock ownership/Corporate boards-employment</td>
<td>No Disclosures</td>
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Estimated Global Meat Production

Million metric tons (1 = 1,000,000,000 kg)

- Poultry
- Pork
- Beef and veal

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<th>Year</th>
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<td>1980</td>
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<td>1984</td>
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<td>1988</td>
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Projections:

Million tons (meat consumed)

- China
- US

USDA Agricultural Projects to 2019 (February 2010). Economic Research Service, USDA
Commonplace: Adverse Respiratory Health

Upper Respiratory Disease:
60-70%: Rhinitis/Sinusitis

Lower Respiratory Disease (6%-50%):
Asthma, Wheeze, Bronchitis, Obstruction, Hypersensitivity pneumonitis (HP)

Chronic bronchitis & COPD: Up to 33%
Highest prevalence: Livestock workers
Pig (15-32%)
Dairy (6.4 to 19%)
Followed by sheep, goat, poultry

Poole. Ann Allergy Asthma Immunol 2012
Slager, et al J Toxicol Environ Health A 2010
Critical Exposure Component(s) Driving Inflammation

- Endotoxin remains important, but does not explain entirety of outcomes.
- Gram positive bacterial components play key role.
- Central role for MyD88 signaling.

Chronic Inflammatory Adaptation-Like Response

- Acute inflammatory response wanes with time, but low-grade chronic inflammation persists.
- Th1/Th17 lung microenvironment with activated macrophages.
- Less IgE mediated disease.
Critical Exposure Component(s) Driving Airway Inflammation to Industrial Animal Farm Pollutants
Industrial Animal Farm Pollutants:

- **Gases**
  - Ammonia, carbon dioxide, hydrogen sulfide
- **Allergens**
- **Pesticides**
- **Inorganic dusts**
  - Particulates, minerals, trace metals
- **Airborne organic dusts**
  - Rich in **gram positive** and gram negative bacteria and other microbes

Tremendous Bacterial Burden in Animal Confinement Facilities

**Culture-dependent:**
~80-98% Gram-positive bacterial burden
(Staphylococcus, Bacillus, Streptomyces, Enterococci)

**Culture-independent: Applied Molecular Approach**
~94% Gram-positive bacterial burden
(Eubacterium, Clostridium, Bacillus, Lactobacillus, Streptococcus)
~Archaebacteria also found

**Chemical marker analysis: Mass Spectrometry**
~High Muramic acid (Peptidoglycan, PGN)
~Increased 3-hydroxy fatty acid (Lipopolysaccharide, LPS)
~Minimal ergosterol (fungi)

Human Studies: Endotoxin Challenges ≠ Barn Challenges

Bronchial hyper-responsiveness to methacholine (PD_{20} mg)

- **Baseline**
- **After LPS challenge**
  - LPS = 53 µg or 200x LPS exposure in pig barn
- **After Pig barn challenge**

Complex (Swine Confinement) Organic Dust:

Settled surface dust

↓

Aqueous Extraction

↓

Sterile filtration (0.2 µM pore)

↓

Organic dust extract (ODE or DE)

---


![Graph showing TNF-alpha (pg/mL) response to different concentrations of organic dust extract.](#)
Lipoproteins, Gram-positive and mycobacterial constituents

TLR1/TLR2

TLR2/TLR6

Gram-negative lipopolysaccharide

TLR4

IL-1, IL-18

Muramyl dipeptide

NOD2

MyD88

IRAK4

IRAK1

NF-κB

MAPK

C57BL/6 strain
Intranasal inhalation technique

CpG DNA
Modest Role for TLR4 Signaling Pathway

Swine Barn Air
Charavaryamath et al., Exp Lung Res 2008

Organic Dust Extract
Bauer et al., Am J Respir Cell Mol Biol 2013.

No difference in airway hyper-responsiveness
Minimal difference in BALF cytokine levels
Minimal difference in lung histopathology
Role for TLR2 Signaling Pathway

No difference observed with:
1. Airway hyper-responsiveness
2. Alveolar inflammation

Modified from Poole, et al. Am J Respir Cell Mol Biol, 2011
TLR9 knockout animal studies

Suggests modest role for TLR9 (CpG agonists) involvement in acute organic dust exposure response

Bauer et al., Am J Respir Cell Mol Biol 2013
NOD2: Negative Regulator of Dust-Induced Inflammatory Outcomes

MyD88 Deficient Mice are Protected from Acute Dust-Induced Airway Inflammatory Response

Bauer et al., Am J Respir Cell Mol Biol 2013
Translation Advances Applied to Adult Farmers

1. Protective effects of TLR2 polymorphisms on lung function among swine workers.
   
   TLR2-16933T/A polymorphism (AA) and TLR2Arg677Trp
   No effect observed with TLR4

2. Increased wheeze with CD14 polymorphisms in older adult farmers
   
   CD14/-159 and CD14/-1619G
   No effect observed with TLR4

Levan TD et al. Am J Respir Crit Care Med 2005
Chronic Inflammatory Adaptation-Like Response to Industrial Animal Farming Exposures

- Less IgE mediated disease.
- Acute inflammatory response wanes with time, but low-grade chronic inflammation persists.
- Th1/Th17 lung microenvironment with activated lung macrophages.
The Hygiene Hypothesis

Raising Swine: High Asthma Prevalence Among Iowa Children

**Lower prevalence of atopy (IgE)**

- Farm - Swine: 34%
- + Farm - Swine*: 26%
- + Farm 1-499 Swine: 43%
- + Farm + Swine: 44%
- + Farm 500+ Swine: 46%
- + Farm + Swine + Antibiotics: 56%

Murine Models Developed to Resemble Adaptation Response Observed in Humans

Lung Macrophage: Important Player

Intranasal Delivery Encapsulated Clodronate Liposomes

Lung Macrophages Deleted

Macrophage Positive
Saline-Liposome (SL-LIP) + Dust

Macrophage Depleted
Clodronate-Liposome (CL-LIP) + Dust

Neutrophils (x10^6)

A Role for T-cells: Th1/Th17

- Influx of CD4+ T-cells
- Th1/Th17 lung polarization
- Confirmed role of T cells in aggregates.
  - T cell deficient mice protected from cellular aggregate development

Organic Dust Exposure

Single Exposure: Robust Inflammation

Repetitive Exposure: Chronic Inflammatory Adaptation Response Decreased IgE-inflammation
Systemic Inflammatory Effects of Inhalant Lung Injury?

Neurologic?

Cardiovascular?

Bone? Osteoporosis?
Overview of Experimental Design

Intranasal instillation

Repeated daily exposures

X 3 weeks

C57BL/6

Calcaneus bone

1. Micro CT analysis
2. Histopathology
Potential (Novel) Therapies

- Supplemental vitamin D beneficial in acute-organic dust exposure:
  - Decreased dust-induced neutrophil influx and chemokine release.
  - Decreased dust-induced trachea epithelial PKC activity.
  - Vitamin D decreased monocyte TLR2 and TLR4 expression and increased NOD2 expression.
Novel Therapies: A potential role for pro-resolving lipid mediators derived from omega-3 fatty acids

Maresin-1 reduced organic dust extract-induced epithelial cell pro-inflammatory consequences in vitro.

Animal studies with maresin-1 are promising.

Nordgren TM et al., Resp Research 2013.
Nordgren TM et al. in submission
Conclusions

- Organic dust environments, particularly large animal industrial farming, are respiratory health risks.
- Farming environments are microbial-rich environments with an emerging role for gram-positive bacterial components.
- Important roles for TLR2, TLR4, TLR9, and NOD2 signaling pathways with a central role for MyD88.
- Activated lung macrophages and Th1/Th17-polarized T cell responses are important.
Many, many thanks to…

My Lab
Angela Gleason
Chris Bauer
Elizabeth Klein
Gregory Golden
Leigh Anderson

Ag Research Group
Debra Romberger
Todd Wyatt
Kristina Bailey
Michael McCaskill
Tricia LeVan
Art Heires
Tara Nordgren

Rheumatology Research Group
Geoff Thiele
Anand Dusad
Michael Duryee
Ted Mikuls
Lynell Klassen

More Thanks to:
Tammy Kielian
William West
Stephen Reynolds
Neil Alexis
Joe Sisson
My Family

Particular thanks to NIEHS, NIOSH, CS-CASH for funding this work.