Allergy, Asthma, and Microbes: Investigating the Hygiene Hypothesis

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February 11, 2012
Faculty Disclosure

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For the 12 months preceding this CME activity, I disclose the following types of financial relationships:

Honoraria received from: None

Consulted for:
  Genentech, GlaxoSmithKline, Healthcare Forecasting Inc., Johnson & Johnson, Merck

Held Common Stock in: None

Research, clinical trial, or drug study funds received from:
  Genentech, GlaxoSmithKline

I will not be discussing products that are investigational or not labeled for use under discussion.
Overview of Argument

- Consistency of data on animal exposure and risk of allergic disease.
- Quantification of “dirt” consumption by children (and adults).
- Microbial content of house dust.
- Gut microbiota effects on immune function (animal studies).
- Linking these observations together into a coherent theory.
Animal Exposure and Allergy Risk

- Cross-sectional survey of rural areas in Austria, Germany, Switzerland (only nationals analyzed)
- 2618/3504 (78%) of 6-13 year old children interviewed about allergic diseases - ISAAC
- Measurement of allergen specific IgE antibodies (n=901)

Farm Animal Exposure And Allergy Risk

Detroit Childhood Allergy-Asthma Study: CAS

- Birth cohort of 833 middle-class children living in suburbs
- Yearly questionnaires concerning home environments plus home visits at 2&4 years
- Evaluation between 6 and 8 years for asthma and allergy

Ownby DR et al  JAMA 2002;288:963-972
First Year Pet Exposure And Allergic Sensitivity: CAS

Ownby DR et al  JAMA 2002;288:963-972
Lung Function And First Year Pet Exposure: CAS

Ownby DR et al  JAMA 2002;288:963-972
Pet Exposure and Total IgE at 18 Years of Age: CAS

All Participants

<table>
<thead>
<tr>
<th>No pets</th>
<th>1 pet</th>
<th>2+ pets</th>
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<tbody>
<tr>
<td>165</td>
<td>314</td>
<td>86</td>
</tr>
</tbody>
</table>

p = .053, p trend = .017

Allergen Sensitized Participants

<table>
<thead>
<tr>
<th>No pet</th>
<th>1 pet</th>
<th>2+ pets</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>176</td>
<td>40</td>
</tr>
</tbody>
</table>

p = .029, p trend = .007

Farm Animal Exposure?

or Pet Exposure?
Meta-analysis of Farm & Pet Exposure vs Allergy Risk

Tse K, Horner AJ. Seminar Immunopath 2008;30:53-62
Role of Unpasteurized Milk

- Multi-center European study (PARSIFAL)
- 14,893 children 5-13 years-of-age
- Farm milk inversely associated with asthma, adj OR = 0.74 (95% CI 0.61-0.88)
- Rhinoconjunctivitis, sensitization to pollen and food mixes also significantly inversely associated with farm milk

Drinking Water, Microbes, and Atopy

- 563 children, 7-16 years, living in Finnish- and Russian-Karelia
- Skin prick tested with 14 common allergens and foods
- Finnish children significantly more sensitization – 48% vs 16%
- Multivariate analysis – microbes in water

Common Features of Most Published Studies

- Exposure impact of animals only in 1st year of life
- Farm animals and household pets have similar impacts
- Reduced risk is not allergen specific
- Animal exposure impacts other diseases of immune dysregulation including Th1 diseases – e.g. IBD
- Impact does not correlate well with –
  - Animal allergen concentration
  - Endotoxin concentration
  - Muramic acid concentration
Microbiota Hypothesis

- Most consistent feature of studies of the hygiene hypothesis is the intensity of exposure to microbes, especially in the gut.

Do Infants Acquire Bacteria from House Dust

- Hand-to-mouth activity in all children
- Well studied by toxicologists
- Studies demonstrate that hand-to-mouth activity relates dust and child concentrations of toxins
Soil and House Dust Ingestion by Children

- Soil and dust ingestion directly related to hand to mouth and object to mouth activity in children.
- Hand to mouth activity is highly variable in children and varies indoors and outdoors.
- Average dust ingestion is 30–100 mg/day for children 6 months – 11 years of age.
- Pica (ingestion of large quantities of soil [~5 gm/day] is relatively common) in children.

U.S. EPA. Child Specific Exposure Factors Handbook 2008
Estimated Bacterial Ingestion from House Dust

- qPCR estimated bacterial load of house dust
- $7.2 \times 10^5$ cells/mg of dust
- Numbers of bacteria ingested by normal children
  - 30 mg = $2.2 \times 10^7$ bacteria/day
  - 100 mg = $7.2 \times 10^7$ bacteria/day

Conceptual Model of How Pets Influence Allergic Disease

- Prenatal Immune Status
- Household Characteristics
- Microbial Community Composition In Home
- Early Immune Response & Development
- Baby/Child’s Gut Microbial Community Composition
- Baby’s Genotype, Season, SES, Upper Resp Infect, Antibiotics, Diet, Activity, Pets, Other Children, Pollutants, Stress
- Persistent Immune Response Phenotype
- Allergic Asthma
Investigating the Conceptual Model: Overarching Hypothesis

That the risks of development of allergy and asthma are influenced by the content of house dust to which a child is exposed in the first years of life.
Sub-Hypotheses

• That the content of house dust shapes microbial community composition in the intestinal tract of infants growing up in the household.

• That microbial communities in the intestinal tract shape the development of immune function.

• That differences in immune function shape the broncho-pulmonary response to exposure to allergens, viruses, bacteria, and/or fungi.
Sub-sub Hypotheses

• That the content of house dust shapes microbial community composition in the intestinal tract of infants growing up in the household.

  - That the microbial content differs in dust samples collected from households associated with different risks of development of allergies and asthma (eg., with vs without pet dogs or cats).

  - That the microbial content differs in stool samples collected from infants growing up in households associated with different risks of development of allergies and asthma.
Bacterial Culture

• Identifies only the “tip of the iceberg”
• Below the surface: >90% of microbes

- difficult to culture species
- “viable but non-culturable” species
- >500 bacterial species in human oral cavity - 60% cannot be cultured
Gene for 16S rRNA

- Found in all prokaryotes
- Structurally conserved regions
  - Permits universal primer design
  - Permits amplification of 16S from all bacterial species
- Variable region
  - Species specific
  - Permits identification
16S rRNA PhyloChip

- High-density oligonucleotide array
- Affymetrix platform
- Standardized production protocols
- Internal QC

- 500,000 probes on array surface
- 300,000 probes dedicated to specific 16S rRNA sequences
- Permits detection of ~8,500 bacterial taxa (60,000 taxa G3)

Deep-Sea Oil Plume Enriches Indigenous Oil-Degrading Bacteria

Tony C. Azam, Jr.1,2, Eric A. Helsel,3 Todd Z. Beranek,3 Gary L. Anderson,3 Yvette M. Piceno,1 Naqviya Singh,3 Janet K. Jensen,3 Alexander Probst,3 Sharon L. Burgin,3 Julian L. Fritsche,2,3 William E. Schaefer,1,2,3 Mark W. Skirrow,3 James H. Tice,2,3 Kristel G. Crua,4 Thoua R. Ana,2 Regina L. Lemosn,2 Dominique G. Juyon,2 Chelsea Spic,2 Jacob Bartelm,2 Manfred Ac,2 Martin J. Stanta,2 Tony Chakar,2 Eric L. Somnath,2 Patrick D’heurde,2 Harvind N. Hupfer,1,2 Shafiq Chiman,2 Zhiqun Lu,2 Jia X. Xiong,2,3 Yu Deng,2 Shiqing Zhou,2,4 Olivia U. Waven2

1-5 The biological activity and expected fate of the oil are determined by the depth and importance of this event. Here, we report that the dispersed hydrocarbons stimulated deep-sea indigenous γ-Proteobacteria that are closely related to known petroleum degraders. Hydrocarbon-degrading genes were detected in the presence of environmental factors that favor the growth of hydrocarbon-utilizing microorganisms. This suggests that the oil is being degraded by indigenous bacteria that are able to use hydrocarbons as a carbon source, thereby reducing the potential for further contamination of the environment.

The 16S rRNA “PhyloChip”

DNA Extracted from a sample.

Amplified rRNA gene using universal primers designed on conserved 16S sequence.

Ampliicon pool fragmented, biotin labeled and hybridized to 16S microarray.

Fluorescence data analyzed and microbes identified.

Microarray stained, washed and scanned.

Fluorescently labeled 16S sequence hybridizes to its complement sequence on the array surface.
Questions

1. Can we detect differences in bacterial communities in house dust from pet vs non-pet keeping households?

2. Can we detect differences in bacterial communities in stool samples from infants raised in pet vs non-pet keeping households?
Reminder: Effects of Pet Ownership on Prevalence of Allergic Sensitivity

Ownby DR et al. JAMA 2002;288:963-972
WHEALS Dust Sample Analysis

Examined 18 household dust samples

6 ≥ 1 dog; 6 ≥ 2 cats; 6 no pets.

Dust samples from households with pets produced sufficient material for PhyloChip analysis.

Only 3 of 6 “no pet” samples produced sufficient material for PhyloChip analysis. (suggests lower bacterial bioburden).
WHEALS Dust Sample Analysis

- 2155 taxa detected across all samples.

- Greatest mean bacterial diversity found in:
  Dog (1521) > Cat (1183) > No pet (976).

- Greatest diversity: 1916 taxa (Dog).

- Least diversity: 878 taxa (No pet).
Hierarchical Cluster Analysis of Bacterial Communities in House

G1 = High Richness & Diversity.

G2 = low Richness & Diversity.

Comparison of bacterial richness:
Dog vs No pet     $p = 0.00453$
Cat vs. No pet    $p = 0.11256$
Cat vs. Dog       $p = 0.05400$
Bacterial Communities in House Dust from Dog vs No-Pet Households.

Fig. 2-3. Comparison of bacterial community A. Richness, B. Evenness and C. Diversity in dog versus no pet samples.
Pet Behavior and Bacterial Populations in WHEALS House Dust Samples

Diversity of bacterial community in house dust related to

• Hours pet allowed out of doors (p < 0.05)

• Hours pet allowed on parents’ bed (p < 0.009)
Bacterial Phyla, Genera, and Species Associated with Pet Keeping

Abundance of 919 taxa differed significantly in G1 vs G2

- Only 6 more abundant in G1 (*Bacillus licheniformis* and *Clostridium botulinum*).

- 903 more abundant in G2; >50% in Proteobacteria Phylum.

- Top 17:
  - All Proteobacteria. Most in Burkholderiales order, which includes many chitinase producing species.
Questions

1. Can we detect differences in bacterial communities in house dust from pet vs non-pet keeping households?

2. Can we detect differences in bacterial communities in stool samples from infants raised in pet vs non-pet keeping households?
Does Pet Ownership Impact House Dust and Stool Microbiota?

- Dog-ownership associated with trend toward greater richness, evenness and diversity in both HD and 6 mo stool samples.
Questions

3. Is stool microbial community composition modified by ingestion of bacteria?
Trial of Infant Probiotic Supplement For Allergy/Asthma Prevention

**Purpose:** To examine the effect of supplementation with *Lactobacillus rhamnosus* for the first 6 mos of life on development of allergic sensitization and of atopic disease at age 3 yrs.

**Design:** DB, PC, prospective study of 275 newborns with an asthmatic parent.

**Outcomes:**
- Clinical evaluation for AD, AR, recurrent wheezing
- Skin testing at 12 and 36 mos.
- Serum IgE, PBMC responses to allergens at 12 and 36 mos.
- Diaper samples to confirm adherence at 1, 3, 6, and 12 months

TIPS Stool Samples

- Total of 16 stool samples from 6 month old infants in the TIPS study analyzed by 16S rRNA PhyloChip
- 100% of samples produced a 16S rRNA signal
- Number of taxa detected: 1984
- Primarily belonging to the Firmicutes, Bacteroides, Actinobacteria, Proteobacteria

*Cox MJ, PLoS One 2010; 5:8745*
Bacterial Community Richness; LGG Abundance.

Hierarchical Cluster Analysis: LGG Abundance Associated with Bacterial Community Structure

Microbial Environment and Development of Asthma

Fig. 1. Clinical and epidemiological variables that impact allergy and asthma development.
Testing the Hypothesis: Microbiomes of Interest

- Bacterial
- Fungal
- Viral
Testing the Hypothesis: Outcomes of Interest

- Development of allergic disease in childhood (allergic dermatitis, asthma).
- Development of markers of pre-disposition to development of allergic diseases (allergic sensitization; severe illness from RTI in 1st year, wheezing illness from RTI in 2nd and 3rd year).
- Development of markers of immune function thought to be related to development of allergic disease (PBMC production of Th1/Th2 cytokines, Treg cell number and function, Th17 cell number and function, DC function.....Best examined in animal models)
The Next Frontier

If we find different bacterial communities, can we identify the genera or species responsible for altering immune function in a way that increases risk for allergies and asthma?

Animal Studies.
Human Studies.
Th-17 Cell Dependence on Intestinal Bacteria in Mice.

Observation: In Lamina propria of C57BL/6 mice from:
- Taconic Farms - Many Th17 cells, Few Treg cells
- Jackson Labs   - Few Th17 cells, Many Treg cells

Approach: Compare intestinal bacterial populations

Findings:
- 766 bacterial taxa; 479 differed between T & J strains
- only 2 differed by >25 fold:
  
  *Lactobacillus murinus* and *Candidatus Arthromitus*  
  (Segmented filamentous bacteria)

Ivanov I, Cell; 139:1-14, 2009
Feeding GF Mice Segmented Filamentous Bacteria Induces Th-17 Cell Expansion

Ivanov I, Cell; 139:1-14, 2009
Gut Microbial Community Composition Affects Immune Function Relevant to Allergy & Asthma.

- Feeding a mix of Clostridium strains (clusters IV and XIV) to SPF BALB/c mice induced expansion of Treg cells in the colonic mucosa, and reduced systemic IgE production after OVA sensitization.
  
  Atarashi, Science 2011; 331:337-341.

- Oral treatment of BALB/c mice with Lactobacillus reuteri induced expansion of Treg cells in circulation, spleen, & mediastinal node, and reduced inflammatory response to OVA challenge in sensitized mice.

  Karimi, AJRCCM 2009; 179:186-93.
Gut Microbial Community Composition Affects Immune Function Relevant to Allergy & Asthma.

- Allergic airway inflammation induced by OVA sensitization and challenge significantly greater in GF mice than in isogenic SPF mice.
- Exaggerated inflammation reversed by colonization of GF mice with gut microbiota from SPF mice.

Herbst T, AJRCCM; 2011; 184:198-205

Gut microbiota regulate immune defense against respiratory tract influenza A virus infection in C57BL/6 mice.

Ichinohe T, PNAS; 2011
Feeding Bacteria or Bacterial Products Affects Response to Viral Respiratory Infection in Humans.

- Twice daily treatment with *L. acidophilus* and *B. animalis* for six months reduced fever, rhinorrhea, cough, antibiotic use, and missed school days in 3-5 y.o. children.


- Oral treatment with a mixture of lyophilized bacterial extracts (OM-85BV) for first 10 days of 3 consecutive months reduced cumulative number of RTI’s and of wheezing episodes per child/yr and reduced duration of wheezing episodes (p < 0.001)

  *Razi, JACI 2010; 126:763-0*
Pets Expose Children to Bacteria
Intimate Exposure to Dirt and Animals in Infancy is not Incompatible with Good Health
Thanks and Acknowledgements.

Henry Ford Hospital
   Kevin Bobbitt PhD
   Christine Cole Johnson PhD MPH
   Al Levin PhD
   Edward L. Peterson PhD
   Ganesa Wegienka PhD, MS
   Kim Woodcroft PhD, MPH
   Edward M. Zoratti MD

Georgia Health Sciences University
   Dennis R. Ownby MD

University of California-San Francisco
   Susan Lynch PhD
   Kei Fujimura, PhD

University of Michigan
   Nicholas Lukacs PhD
QUESTIONS?