Infant Immune Responses and the Microbiome

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Microbial Culture does not Reflect the True Diversity of Microorganisms Present
Molecular Approaches to Interrogate Microbial Dark Matter

DNA
- 16S rRNA
- ITS2 rRNA
- ShotSeq metagenomics
- Composition
- Functional capacity

RNA
- Metatranscriptomics
- Gene expression

Protein
- Metaproteomics
- Catalytic functions

Metabolites
- Metabolomics
- Metabolic activity
# The Human Microbiome

## Gut Microbiota Functions

<table>
<thead>
<tr>
<th>Influences</th>
<th>Neurologic</th>
<th>Psychiatric</th>
<th>Respiratory</th>
<th>Cardiovascular</th>
<th>Gastrointestinal</th>
<th>Hepatic</th>
<th>Metabolic</th>
<th>Oncologic</th>
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<td>Immune maturation &amp; homeostasis</td>
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<td>Host cell proliferation</td>
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<td>Vascularization</td>
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<td>Neurologic signaling</td>
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<td>Pathogen burden</td>
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<td>Intestinal endocrine functions</td>
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<td>Bone density</td>
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<td>Energy biogenesis</td>
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| Biosynthesis                                   |            |             |             |                |                  |         |           |           |
| Vitamins                                       |            |             |             |                |                  |         |           |           |
| Steroid hormones                               |            |             |             |                |                  |         |           |           |
| Neurotransmitters                              |            |             |             |                |                  |         |           |           |

| Metabolism                                     |            |             |             |                |                  |         |           |           |
| Branched-chain & aromatic amino acids          |            |             |             |                |                  |         |           |           |
| Dietary components                             |            |             |             |                |                  |         |           |           |
| Bile salts                                     |            |             |             |                |                  |         |           |           |
| Drugs                                          |            |             |             |                |                  |         |           |           |
| Xenobiotics                                    |            |             |             |                |                  |         |           |           |

Children who exhibit early life allergic sensitization are at significantly higher risk of developing asthma in childhood.
Asthma is Prevalent in Westernized Nations


Graham-Rowe, D. Nature 479, S2–
Risk Factors for Childhood Atopic Asthma Also Impact the Microbiome

- Factors associated with childhood atopy development:
  - Lack of maternal exposure to livestock or furred pets during pregnancy
  - Formula feeding
  - Caesarian section delivery
  - Maternal antibiotic use during pregnancy
  - Early life antibiotic exposure

Early life fecal enrichment of *Clostridium difficile* or *Escherichia coli* is associated with childhood allergy

Penders et al. Journal of the British Society for Allergy and Clinical Immunology 2006;36:1602-8.
Kalliomaki et al. JACI. 2001;107:129-34.
Fujimura et al, ERAIT 2010
Risk Factors for Childhood Atopic Asthma Also Impact the Microbiome

Faith's Phylogenetic Diversity Index

Time of sample collection [month]

Month: 0, 1, 3, 6, 9, 12

K' = 0.564
p = 0.001

Toll-like receptors
Dendritic cells
T cells (immunogenic)
IgA

Pre-natal  Birth  Adult

Rauch et al, unpublished data
In Westernized Nations Humans Spend a Disproportionate Amount of Time in the Built Environment

86.9%
(68.7% in residence)

7.6%

5.5%

Klepeis et al. 2001 J Expo Anal Env Epid 11:231-52
Hypothesis: Early life house dust microbial exposures are related to atopy and/or allergic wheeze outcomes in childhood.
Urban Environment & Childhood Asthma (URECA) Study

• Prospective cohort study of full-term infants born in low-income, inner-city urban US neighborhoods

• Baltimore, Boston, New York City, St. Louis

• Total of 609 families enrolled 2/05 – 3/07

• House dust collected during the first year of infants life (3 months of age), and atopy and recurrent wheezing defined at age three years

• Bacterial microbiota determined using 16S rRNA PhyloChip
Atopic Wheezers Experience Distinct Bacterial Exposures in Early Life

Protective Taxa

Enriched in Neither (vs Atopic):
Bifidobacteriaceae, Prevotellaceae, Lachnospiraceae, Ruminococcaceae, Veillonellaceae, Dethiosulfovibrionaceae

Enriched in Neither (vs Both):
Acidobacteriaceae, Bacteroidaceae, Blattabacteriaceae, Porphyromonadaceae, Prevotellaceae, Rikenellaceae, Catabacteriaceae, Enterococcaceae, Lachnospiraceae, Ruminococcaceae, Veillonellaceae, Gemmatimonadaceae, Planctomycetaceae, Dethiosulfovibrionaceae, Erysipelotrichaceae

Atopic and atopic wheezers exposed to fewer and distinct bacteria

Early-life Dog Exposure is Protective Against Childhood Allergic Disease Development

- Exposure to dogs, and to a lesser extent cats, in early life is protective against childhood allergic disease development

High-risk children have altered microbial exposure; fewer bacterial species, enrichment for fungi.

**Children from homes with no pets exposed to less bacteria, wider range of fungi**

Can Distinct House Dust Exposures Influence Airway Mucosal Immunity via the Gut Microbiome?

Dog VS No pets

daily gavage w/dust 2.5mg/100µl

I.T. CRA I.T. CRA I.T. CRA EP

D-7 D0,1,2 D14 D20, 22 D23

House Dust Exposure Alters Airway Response to Allergen Challenge

Gut Microbiome is Distinct in Protected Mice

Pet ownership modifies household microbial exposure, impacts gut microbiome composition and conveys protection against allergic airway inflammation.

- ~100 taxa discriminated protected and unprotected animals
- *Lactobacillus johnsonii* was amongst the top discriminatory taxa


(Image courtesy of Kathryn Cross, IFR)
Oral *Lactobacillus* Supplementation Protects against Airway Allergen Challenge
Viable *Lactobacillus* Supplementation is Necessary for Protection against Viral Respiratory Infection

Oral supplementation with *L. johnsonii* in the RSV model

- Daily gavage with $10^7$ CFU/100 µl *L. johnsonii*
- I.T. RSV 3.7 x $10^6$ PFU in 40 µl
- Gavage with $10^7$ *L. johnsonii* 2x per week
Viable *Lactobacillus* Supplementation is Necessary for Protection against Viral Respiratory Infection

Bronchial hyper-responsiveness

Airway cytokine responses

Viable *Lactobacillus johnsonii* provides limited protection against allergic airway inflammation; is protective against asthmagenic virus
HOW ?
High-Fiber Diet Decreases Susceptibility to Allergic Airway Inflammation

High-Fiber Diet Decreases Susceptibility to Allergic Airway Inflammation

Gut microbiome

Control diet

High-fiber diet

- Erysipelotrichaceae
- Ruminococcaceae
- Lactobacillaceae
- Prophyromonadaceae
- Bacteroidiales
- Bifidobacteriaceae

Airway response

- IL-4 (pg ml⁻¹)
- IL-5 (pg ml⁻¹)
- IL-17A (pg ml⁻¹)
- IL-13 mRNA (IL-13/β-actin ratio)

Increased concentrations of microbial-derived SCFAs specifically propionate associated with protection against allergic sensitization

High fiber diet product, specifically short-chained fatty acid, conveys protection against allergic airway inflammation

Trompette et al Nature Medicine. 20, 159-166 (2014)
Is the Gut Microbiome Related to Allergy and Asthma in Humans?
Gut Bacterial Community Dysbiosis Evident in 3-Month Old Atopic- Wheezers

Arrieta et al., Sci Transl Med 2015;7:307ra152
Atopic wheezers had depletion of key bacteria from the gut microbiome, decrease of short-chain fatty acid and altered metabolite profile.
To realize the potential of the gut microbiome we must understand it both at an ecological and a mechanistic level.
“Sociomicrobiology” is Fundamental to Microbial Interactions and Microbiota Development

• Inter-species communication and metabolic cross-feeding dictates species burden and behavior
  • Hypothesis: Microbial inter-species interactions deterministically lead to reproducible patterns of microbial co-association

• Pioneer colonizers influence ecosystem conditions and subsequent patterns of species accumulation

Hypothesis

Compositionally and functionally distinct gut microbiota exist in neonates and are related to risk of allergic sensitization and asthma in childhood
Wayne County Health, Environment, Allergy & Asthma Longitudinal Study (WHEALS)

- Birth cohort - sample collection commenced in 2003
- Socio-economic and racially diverse (50% minority)
- 298 independent infant stool (0-11 months) samples targeting:
  - Neonates (median age 35 days; n=130)
  - Infants (median age 201 days; n=168)
- Population-based cohort, cross-sectional study
- Gut microbiota composition examined using 16S rRNA V4 and ITS2 rRNA regions (Illumina MiSeq platform)
Infants Stratified into Two Age-dependent Gut Microbiota States

IGM age is significantly different (Wilcoxon rank sum, p=0.0257)

R²=0.34; p<0.001

Neonates Stratified Into Three Age-independent Gut Microbiota States

$R^2=0.09, p<0.001$

PC1 (9%)
PC2 (5%)
PC3 (4%)
NGM1
NGM2
NGM3

Kruskal-Wallis $p=0.256$

NGMs are Associated with a Significantly Different Relative Risk of PM-Atopy and Asthma

Inter-Kingdom Dysbiosis Characterizes NGM3

- NGM3 is depleted of key bacterial species e.g. *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Lactobacillus ruminus*
- Highly enriched for *Candida* and *Rhodotorula*

NGM 3 Microbiota Perturbations are Deterministically Linked to Metabolic Dysfunction in the Neonatal Gut

PICRUST Predicted Functional Capacity

Metabolism
- Amino Acid
- Carbohydrates
- Cofactors & Vitamins
- Energy
- Lipid
- Nucleotide
- Xenobiotics

NGM 3 Microbiota Perturbations are Deterministically Linked to Metabolic Dysfunction in the Neonatal Gut
Hypothesized Metabolism of Linoleic Acid by NGMs

Low-risk NGMs

Produce anti-inflammatory metabolite (PGE1)

Dihomo-\(\gamma\)-linolenate

Linoleoyl-CoA desaturase

Linoleic acid

Block pro-inflammatory metabolite (AA-derived 5-lipoxygenase metabolites)

High-risk NGM

Produce 12-13, DiHOME

NGM 3 Microbiota Perturbations are Deterministically Linked to Metabolic Dysfunction in the Neonatal Gut

Table 10: Procrustes analyses of 16S rRNA phylogeny, PICRUSt and metabolomic datasets. Results from Procrustes analyses indicate that 16S rRNA phylogeny, PICRUSt and metabolomic data is highly and significantly correlated.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>$r^*$ ($M^2$)</th>
<th>$P$-value</th>
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<tbody>
<tr>
<td>16S rRNA versus PICRUSt</td>
<td>0.72 (0.48)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>16S rRNA versus Metabolomics</td>
<td>0.87 (0.24)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PICRUSt versus Metabolomics</td>
<td>0.66 (0.56)</td>
<td>0.010</td>
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</table>

*r* = correlation between data sources. Unweighted UniFrac distance used for 16S rRNA; Canberra distance used for PICRUSt and Metabolomics.
Ex vivo DC/T-cells Assay to Assess Immunostimulatory Capacity of NGM Fecal Water

Dendritic Cell + Filter sterile NGM1 or NGM3 fecal water
2 day incubation

DC | Naïve T-cell

Phenotype
CD4+
- Th1: IFN-γ
- Th2: IL-4
- Th17: IL-17
- Treg: CD25, Foxp3

Wash
5 day co-culture

NGM3 Sterile Fecal Water Exposure Induces a Pro-allergic Response and Suppresses T-regulatory Cells

Luminal environment of the high-risk NGM neonate is pro-inflammatory, and this may interfere with gut microbial development

Can we Identify Specific NGM3-associated Metabolites that Recapitulate Features of Immune Dysfunction
Weighted Correlation Network Analysis Identifies Metabolic Modules in the Neonatal Gut

A Single Metabolic Module is Associated with NGM3

NGM 3 Microbiota Perturbations are Deterministically Linked to Metabolic Dysfunction in the Neonatal Gut

12,13 DiHOME Suppresses T-regulatory cell Populations 
ex vivo

Products associated with a perturbed neonatal gut microbiome can drive immune dysregulation associated with allergic asthma.

Summary

• Early life environmental microbial exposures are related to childhood asthma development

• Gut microbiome composition and metabolic function influences airway mucosal immunity

• Distinct neonatal gut microbiota and metabolic re-programming are associated with immune dysfunction of high risk children
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