How Gut Microbes Alter the Risks of RSV Infection and Asthma in Mice and Men

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Disclosures

• No commercial financial relationships
• No discussion of off label drug use
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• Other potential conflicts: none

Learning Objectives

• Understand why *Lactobacillus johnsonii* maybe a useful probiotic for asthma prevention
• Recognize the types of metabolic products that maybe altered with *Lactobacillus johnsonii* supplementation
• Describe how microbial metabolic products may influence development and function of immune system
Distinct Neonatal Gut Microbiologic States Related to Allergen Specific IgE

<table>
<thead>
<tr>
<th>Outcome</th>
<th>NGM2 vs NGM1</th>
<th>NGM3 vs NGM1</th>
<th>NGM3 vs NGM2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopy</td>
<td>1.43 (0.73-2.81)</td>
<td>2.94 (1.43-6.09)</td>
<td>3.06 (1.36-6.93)</td>
</tr>
<tr>
<td>p</td>
<td>p=0.3</td>
<td>p=0.004</td>
<td>p=0.048</td>
</tr>
<tr>
<td>Asthma</td>
<td>0.87 (0.40-2.03)</td>
<td>2.85 (1.09-7.98)</td>
<td>1.36 (1.00-10.0)</td>
</tr>
<tr>
<td>p</td>
<td>p=0.82</td>
<td>p=0.033</td>
<td>p=0.504</td>
</tr>
</tbody>
</table>

Sterile Water from Stools of Children in Different Neonatal Gut Microbiologic States Alter DC:CD4+ Cell Interactions

<table>
<thead>
<tr>
<th>PBS</th>
<th>NGM1</th>
<th>NGM3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ IL4+ %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL4+ pg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ CD25+Foxp3+ %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Metabolites Vary by Neonatal Gut Microbiologic State and 12,13 DiHOME ReducesSuppressor Cell Production

Fujimura KE, et al. Nature Medicine 2016 (advance on line publication)
House Dust Administration During Allergen Challenge

- Dust was sieved and weighed out to 25 mg/tube and suspended in saline (1 ml) on the day of treatment (dust was stored at 4C dry until use).
- Each mouse was given 100 ul of the Dust suspension immediately after suspension.
- Cockroach Allergen is an endotoxin-free skin test antigen preparation given at 5 ug/treatment.

Fujimura KE, et al. PNAS 2014;111:805-810

Alteration In Pulmonary Responses In Lungs Of Mice With Dust-induced Microbiome Changes

Fujimura KE, et al. PNAS 2014;111:805-810

Why Lactobacillus johnsonii

Fujimura KE, et al. PNAS 2014;111:805-810
Questions about *Lactobacillus johnsonii* and Metabolites

- **Hypothesis:** Supplementation with *L johnsonii* protects against RSV infection through changes in circulating metabolic microenvironment which concurrently impacts both airway mucosal responses and bone marrow derived immune precursor cell populations.
- Metabolic changes with *L johnsonii* supplementation
- Effects of altered metabolites on immune response

Mouse Experimental Plan

*Fonseca W, et al. submitted August, 2016*
Effect of *L. johnsonii* Supplementation on RSV Infections at Days 2 & 4

**A** Cell Cytokine Production LDLN

**B** RSV Protein Expression


Changes with *L. johnsonii* Supplementation

Airway epithelia cells (AEC) infected with RSV in vivo showed no difference in cytokines between supplemented and control mice

Prior to RSV challenge supplemented mice showed increased Treg cell numbers in lung and colon.


Cytokine Production Day 8 Post RSV Infection

Alterations of Bone Marrow Derived Dendritic Cells

Systemic Metabolic Changes

<table>
<thead>
<tr>
<th>Super Pathway</th>
<th>Sub Pathway</th>
<th>Biochemical Name</th>
<th>Metabolite Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xenobiotic</td>
<td>Food Component</td>
<td>methyl-alpha-glucopyranoside</td>
<td>1.69</td>
</tr>
<tr>
<td>Lipid</td>
<td>Lysolipid</td>
<td>20:4n6-arachidonoylglycerophosphocholine*</td>
<td>1.32</td>
</tr>
<tr>
<td>Lipid</td>
<td>Polyunsaturated Fatty Acid (n3 and n6)</td>
<td>docosahexaenoate (DHA; 22:6n3)</td>
<td>1.31</td>
</tr>
<tr>
<td>Lipid</td>
<td>Polyunsaturated Fatty Acid (n3 and n6)</td>
<td>1-docosahexanoylglycerophosphocholine (22:6n3)*</td>
<td>1.26</td>
</tr>
<tr>
<td>Lipid</td>
<td>Lysolipid</td>
<td>20:4n6-arachidonoylglycerophosphocholine (20:4n6)*</td>
<td>1.19</td>
</tr>
<tr>
<td>Lipid</td>
<td>Chemical</td>
<td>2-hydroxyisobutyrate</td>
<td>1.18</td>
</tr>
<tr>
<td>Lipid</td>
<td>Lysolipid</td>
<td>1-palmitoylglycerophosphocholine (16:0)</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Based on examination of >400 metabolites; 2 experiments, 5 mice per experimental group; 56 alterations

Effects of DHA and Butyrate on RSV Infected Bone Marrow Derived Dendritic Cells
Gut Microbiome and Inflammation

- Omega-3 fatty acids (n-3 PUFA) are essential nutrients
  - Eicosapentaenoic acid (EPA) and docosahexanenoic acid (DHA)
  - Bacteria (cold water marine) can synthesize n-3 PUFA
  - Transformed in resolvins and protectins
  - Members of the resolvin E family are generated via the aspirin:COX2 pathway

Over Production of Omega-3 Fatty Acids

Pre- and Probiotic Supplementation Prevents RV Infections in Preterm Infants

- Randomized, double-blind, placebo controlled trial
- 94 preterm infants: ≥32+0 and ≤36+6 weeks, birth weight >1500 g
- Turku University Hospital, Turku, Finland
- Oral prebiotics: galacto-oligosaccharide and polydextros mixture 1:1, or
- Probiotic: *Lactobacillus rhamnosus* GG, or
- Control: microcrystalline cellulose
- Days 3 to 60 of life

Pre- and Probiotic Supplementation Prevents RV Infections in Preterm Infants

- Supplement added to 10 ml of breast milk
- Follow-up visits at 1, 2, 4, 6, & 12 months plus telephone call at 9 months.
- Parent reports of behavior, sleeping, fussing, crying, irritability, feeding, vomiting, stool consistency & frequency, infections, medications
- Diary of physician visits and medications
- Primary outcome: respiratory tract infection by nasal swab and nucleic acid testing
- Secondary outcomes: RTI symptoms

Prebiotic
Probiotic
Control

Details
Virus detected in 96% of cases
Rhinovirus 80%
2 viruses in 20, 3 in 3 and 4 in 1 case
RV infections by Tx vs placebo
Prebiotic RR = 0.31
(95% CI 0.14-0.66, P=0.003)
Probiotic RR = 0.49
(95% CI .24-.66, P=0.051)

Conclusions
A single bacterial species supplement can significantly alter serum metabolic profiles in mice
Altered metabolic profiles in mice are associated with protection from both allergen- and RSV-induced lung disease
Among the metabolic alterations are increased fatty acids with known anti-inflammatory activity (ω-3 PUFA): EPA, DPA, DHA
Alterations of gut produced fatty acid metabolites results in reduction of Th2 immune responses and increased Treg responses
Conclusions

• In a trial of human premature infants both a prebiotic and a probiotic significantly reduced the total number of acute viral respiratory infections and specifically rhinovirus infections in premature infants