Microbiome, Immune function, and murine pulmonary disease

CURES Symposium:
Addressing the Asthma and Allergy Epidemics
October 7, 2015

Nick Lukacs, PhD
Godfrey Dorr Stobbe Professor of Pathology
University of Michigan Medical School
T cell maturation and differentiation depends upon Immune environments

TLR3  TLR7  IL-12  IFNα/β

Cytokine Mileau  Transcription Factor

IL-12  →  T-bet  →  TH1  ←  IFNγ  IL-1

IL-4  →  GATA3  →  IL-4  IL-5  IL-13

TGFβ  →  RORγt  →  TH17  ←  IL-17  IL-21  IL-22

TGFβ  →  FoxP3  →  TReg  ←  IL-10  TGFβ

IL-12  + Anti-viral immunity
- Airway damage

IL-4  IL-5  IL-13  + Anti-parasitic,
- allergy, asthma

IL-17  IL-21  IL-22  + Anti-bacterial immunity,
- autoimmunity

IL-10  TGFβ  + Tolerance to inert antigens
- Reduced pathogen response

IL-4- IgE → Mast cell activation
IL-5- Eosinophilia → airway damage and fibrosis
IL-13- Goblet cell metaplasia → mucus and airway obstruction
Factors that affect the gut microbiome

Commensal bacteria

Protection
- Mucosal barrier function
- Treg cell development

Development and modulation of the host immune responses

Metabolism

Pathobionts

The resulting overgrowth of the pathobiont may cause inflammation and bleeding of the lining of the colon.

Obesity
Cancer

Adapted from Environ Health Perspect; DOI:10.1289
Infant’s Environment shapes the Microbiome

• Natural vs. Cesarean section birth
• Bottle vs. Breast feeding
• Timing and type of solid food introduction
• Antibiotic use
• Vitamin and nutrition
• Household exposure- high % of early life

Man's best friend? The effect of pet ownership on house dust microbial communities.
- Increase in bacterial diversity and a decrease in fungal species

Dust-exposed mice have a modified response in cockroach allergen (CRA) model.

**A.**
- Black: Allergen
- Light Grey: Allergen + non-Pet Dust
- Dark Grey: Allergen + Pet Dust

**C. Gob5 expression**
- Fold increase over naive

**D. Total IgE**
- ng/mL serum

---

Control CRA NP + CRA D+CRA
Bacterial diversity in Pet dust supplemented animals

PNAS 111:805-810
Lactobacillus Supplementation

- Colonic contents of 4 mice
- *Lactobacillus* isolation media
- Sequenced 6 isolates per mouse
- Twenty one isolates yielded high quality full length 16S rRNA sequence—All were *L. johnsonii*
- 99% coverage and 99% homology to expected *Lactobacillus* species
- Batch culture
- Standardized (1 x 10⁷ CFU) supplements in glycerol
Lactobacillus johnsonii supplementation protects asthmatic mice

PNAS 111:805-810
Respiratory Syncytial Virus (RSV)

- Leading cause of respiratory illness and hospitalization in infants
- Airway epithelial damage
- Leads to long-term Respiratory disease
- Goblet cell hypertrophy, mucus hypersecretion;
- Th2 and Th17 cytokine production;
- Associated with increased Asthma
- During RSV infection-Tregs control the magnitude of cellular immune responses. (Brincks EL, J. Immunology, 2013)

Viral infections during infancy → Asthma?
Viable vs heat killed bacteria
1 x 10^7 CFU daily supplement – 7 days

RSV (line 19) infection on day 8 of treatment protocol

Outcome measurements
1. Airway responses – histology
2. Immune responses – Th2, IFNg, muc5AC Gob5
L. Johnsonii supplementation alters RSV-induced pathophysiology
Dendritic Cells (DC) prime the adaptive immune response

Bone marrow DC are altered in L. johnsonii exposed animals in Response to RSV.
Microbiome can determine metabolite production available to alter immune function
Upregulation of plasma metabolites in animals supplemented with *L. johnsonii*

- Animals were supplemented with 1 X 10^7 cfu of *L. johnsonii* by oral gavage for 7 days and plasma metabolite levels were assessed.

- Supplemented animals were then infected with RSV. After 2 days of RSV infection plasma from sacrificed animals (5/group) was harvested and the metabolite levels compared to supplemented mice at day 0 prior to infection.
<table>
<thead>
<tr>
<th>Sub Pathway</th>
<th>Biochemical Name</th>
<th>PBS</th>
<th>L. johnsonii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine, Isoleucine and Valine Metabolism</td>
<td>alpha-hydroxyisovalerate</td>
<td>1.0314</td>
<td>1.2644</td>
</tr>
<tr>
<td></td>
<td>isoleucine</td>
<td>0.95</td>
<td>1.3852</td>
</tr>
<tr>
<td></td>
<td>3-methyl-2-oxovalerate</td>
<td>0.7178</td>
<td>1.8789</td>
</tr>
<tr>
<td></td>
<td>2-hydroxy-3-methylvalerate</td>
<td>1.1784</td>
<td>1.6114</td>
</tr>
<tr>
<td></td>
<td>valine</td>
<td>0.9468</td>
<td>1.3642</td>
</tr>
<tr>
<td></td>
<td>3-methyl-2-oxobutyrate</td>
<td>0.8829</td>
<td>1.5879</td>
</tr>
<tr>
<td></td>
<td>3-hydroxyisobutyrate</td>
<td>1.0203</td>
<td>1.8043</td>
</tr>
<tr>
<td></td>
<td>alpha-hydroxyisocaproate</td>
<td>0.7684</td>
<td>1.8758</td>
</tr>
<tr>
<td>Long chain fatty acids</td>
<td>myristoleate (14:1n5)</td>
<td>1.0156</td>
<td>1.6242</td>
</tr>
<tr>
<td></td>
<td>palmitate (16:0)</td>
<td>0.8279</td>
<td>1.2426</td>
</tr>
<tr>
<td></td>
<td>palmitoleate (16:1n7)</td>
<td>0.9297</td>
<td>1.9098</td>
</tr>
<tr>
<td></td>
<td>10-heptadecenoate (17:1n7)</td>
<td>0.9143</td>
<td>1.6254</td>
</tr>
<tr>
<td></td>
<td>oleate (18:1n9)</td>
<td>0.9946</td>
<td>1.5634</td>
</tr>
<tr>
<td></td>
<td>cis-vaccenate (18:1n7)</td>
<td>1.1388</td>
<td>1.6774</td>
</tr>
<tr>
<td></td>
<td>10-nonadecenoate (19:1n9)</td>
<td>0.9574</td>
<td>1.7259</td>
</tr>
<tr>
<td>Polyunsaturated Fatty Acid (n3 and n6)</td>
<td>stearidionate (18:4n3)</td>
<td>0.9092</td>
<td>2.0629</td>
</tr>
<tr>
<td></td>
<td>eicosapentaenoate (EPA; 20:5n3)</td>
<td>0.9697</td>
<td>1.6829</td>
</tr>
<tr>
<td></td>
<td>docosapentaenoate (n3 DPA; 22:5n3)</td>
<td>1.0019</td>
<td>2.1902</td>
</tr>
<tr>
<td></td>
<td>docosahexaenoate (DHA; 22:6n3)</td>
<td>1.0062</td>
<td>1.4737</td>
</tr>
<tr>
<td></td>
<td>linolenate [alpha or gamma; (18:3n3 or 6)]</td>
<td>0.9729</td>
<td>1.8151</td>
</tr>
<tr>
<td></td>
<td>docosapentaenoate (n6 DPA; 22:5n6)</td>
<td>0.8102</td>
<td>1.527</td>
</tr>
<tr>
<td></td>
<td>dihomo-linoleate (20:2n6)</td>
<td>0.8467</td>
<td>1.6506</td>
</tr>
<tr>
<td></td>
<td>mead acid (20:3n9)</td>
<td>0.6505</td>
<td>2.1117</td>
</tr>
<tr>
<td>Fatty acid Metabolism</td>
<td>myristoylcaritnine</td>
<td>0.929</td>
<td>1.8567</td>
</tr>
<tr>
<td></td>
<td>palmitoylcaritnine</td>
<td>0.8664</td>
<td>1.5805</td>
</tr>
<tr>
<td></td>
<td>stearoylcaritnine</td>
<td>1.0211</td>
<td>2.2988</td>
</tr>
<tr>
<td></td>
<td>oleoylcaritnine</td>
<td>1.0648</td>
<td>1.5524</td>
</tr>
<tr>
<td></td>
<td>myristoleoylcaritnine*</td>
<td>1.0617</td>
<td>1.6484</td>
</tr>
<tr>
<td>Fatty Acid, Monohydroxy</td>
<td>2-hydroxyoctanoate</td>
<td>0.7521</td>
<td>1.6325</td>
</tr>
<tr>
<td></td>
<td>2-hydroxydecanoate</td>
<td>0.6441</td>
<td>2.0658</td>
</tr>
<tr>
<td></td>
<td>3-hydroxyoctanoate</td>
<td>1.0549</td>
<td>1.9832</td>
</tr>
<tr>
<td></td>
<td>3-hydroxydecanoate</td>
<td>1.1702</td>
<td>2.1847</td>
</tr>
<tr>
<td></td>
<td>3-hydroxylaurate</td>
<td>1.2756</td>
<td>1.9255</td>
</tr>
<tr>
<td></td>
<td>3-hydroxymyritate</td>
<td>1.072</td>
<td>1.6838</td>
</tr>
<tr>
<td></td>
<td>5-hydroxyhexanoate</td>
<td>1.624</td>
<td>0.9847</td>
</tr>
<tr>
<td></td>
<td>12-HODE + 9-HODE</td>
<td></td>
<td>0.3099</td>
</tr>
</tbody>
</table>
Alteration of RSV-induced DC activation by plasma from *L. johnsonii* supplemented mice

- BMDC were pre-incubated with plasma from supplemented animals at day 2 of RSV infection.
- DC were infected with RSV for 24 hrs and assessed for cytokine expression.
- Similar to the response of BMDC from supplemented mice, the plasma *from L. johnsonii* supplemented mice induced higher cytokine production.
Environment, Microbiome, Metabolic activity, and immunity

- Environmental influence and exposure
- Early influence may be crucial for establishing the appropriate immune environment.
- Interface of genetics with the environment has a contributory role in disease susceptibility and severity.

_Brestoff and Artis, Nat. Immun., 14:676, 2013_

**Susceptibility to chronic disease**

- Environmental influence and exposure
- Early influence may be crucial for establishing the appropriate immune environment.
- Interface of genetics with the environment has a contributory role in disease susceptibility and severity.
MAAP Collaboration
NIH, NIAID- P01AI089473