The role of Gut Microbiota in Allergic Disease

Christina E West
MD, PhD
A/Professor, Pediatric allergist
Clinical Sciences, Pediatrics
Umeå University, Sweden
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I receive royalties from UptoDate
• Development of early life gut microbiota and immune tolerance

• Dysbiosis in early life microbiota and allergic diseases

• Current evidence on pre/pro/synbiotics for allergy management

• Results from the ASSIGN trial
Microbial imprinting may start in utero

- DNA from bacterial taxa in the feto-placental unit
- Microbes may travel via the blood stream from the oral cavity to the placenta (Aagard et al, Sci Transl Med 2014)
- Translocation over the gut epithelium also suggested
- Transmission of administered labeled bacterial strains from the mother to her offspring in mice (Jimenez et al, Res Microbiol 2008)
**Postnatal establishment of the gut microbiota**

- **Anaerobes** (Bifidobacteria, Bacteroides, Clostridium spp)
- **Aerobes and facultative anaerobes** (E. coli, Enterococcus spp, α-hemolytic streptococci, Staphylococcus aureus)
- Unculturable bacteria
- Increase in microbial diversity

- Breastfeeding
- Weaning
- Complementary foods

- Delivery/Birth
- Age (yrs) 3

**Genetics and primary exposure**

*Modified from Salminen S, 2005*
Predictable microbiome development

- The early gut microbiota abundant in bifidobacteria
- Microbes that characterize early stages of development are more capable of metabolizing nutrients associated with breast feeding
- Later stages have a gut microbiota enriched in genes that can help to digest solid foods

Caesarean section delivery

↑ Staphylococcus

↑ Clostridium difficile

↓ Bacteroides

↓ Diversity

Vaginal delivery

↑ Lactobacillus

↑ Prevotella

↑ Sneathia

• Caesarean section delivery associated with increased risk of allergic and autoimmune disease (Kristensen K et al, JACI 2016;137:587-90)


Maternal gut
Bifidobacterium
Bacteroides
E. coli

Breastfeeding, vagina
Lactobacillus
Streptococcus

Skin
Staphylococcus

Environment/food
Clostridium difficile
Klebsiella
Enterobacter

Gut microbiota changes faster than our immune system can adapt to


Homeostasis

• Balance between the gut microbiota and the host

• Major functions of gut microbiota
  – Metabolic
    • Fermentation of non-digestible dietary residue
  – Trophic
    • Gut integrity
    • Development of the immune system
  – Protective
    • Protects against pathogens

Dysbiosis

• Gut microbial imbalance
  – May impact both composition and function

• Dysbiosis has been associated with development of allergic diseases in infants and children

• Microbiota establishment is influenced by diet and environmental exposures perinatally and in childhood
• Parallels the development of innate and adaptive immune pathways
  - Short-chain fatty acid production and induction of Treg cells
• Low biodiversity/dysbiosis
  - IgE production and pro-inflammatory responses

Dysbiosis in allergic disease

- Reduced relative abundance (RA) and α-diversity of Bacteroidetes in infancy before onset of IgE-associated eczema and asthma
  
  *Abrahamsson, Jenmalm et al JACI 2012 and Clin Exp Allergy 2014*

- Reduced RA of *Ruminococcaceae* at 1yr, 5 times more likely in food-sensitized infants in the CHILD cohort study
  
  *Azad, Kozyrskyj et al Clin Exp Allergy 2015*

- Reduced RA of *Ruminococcaceae* at 1 week preceded onset of IgE-ass eczema; the RA of *Ruminococcus* was inversely correlated with inflammatory responses
  
  *West, Prescott et al, Clin Exp Allergy 2015*
Toll-like receptors (TLRs)- ancient “gate-keepers” in innate immunity

- Expressed on epithelial and endothelial cells, leukocyte subsets in blood
- Sense conserved structural components of microbes
- T regulatory cells (Tregs) recently shown to express TLRs
- TLR activation can increase or decrease the suppressor activity of T regulatory cells, thus providing an important link between innate and adaptive immunity
Innate immune responses in allergic disease

Increased inflammatory responses following Toll-like receptor (TLR)-activation

*Tulic JACI 2011;122:391*

Immature T-helper 1 (Th1) function

*Tulic JACI 2011;122:391*

Immature T regulatory function

*Smith JACI 2008;121:1460*

*Schaub JACI 2008;121:1491*
Lower abundance of *Ruminococcaceae* in food sensitization and IgE-associated eczema

A reduction of potentially immune-modulatory bacteria (ruminococci) is associated with aberrant innate immune responses and increased risk of atopic eczema

*West et al, 2015*

A reduction in the abundance of *Ruminococcaceae* at 1 yr, was 5 times more likely in food-sensitized infants, independently of

- Delivery mode
- Breastfeeding
- Antimiotics exposure

*Azad et al, 2015*

- *Ruminococci produce bacteriocins, e.g. Ruminococcin A, which can inhibit the development of Clostridium species*

- *Ruminococci can degrade fiber, increase SCFA production*
Early gut microbiome composition associated with resolution of CMA

- Consortium of Food Allergy observational study (N=226 children, enrolled in infancy)
- CMA (IgE-mediated)
- Stool sampled at entry
- Followed until 8 years
- Milk allergy resolved in 128 children (56.6%) by 8 years of age

Supinda Bunyavanich, Nan Shen, Alexander Grishin, Robert Wood, Wesley Burks, Peter Dawson, Stacie M. Jones, Donald Y.M. Leung, Hugh Sampson, Scott Sicherer, Jose C. Clemente

Early-life gut microbiome composition and milk allergy resolution

Journal of Allergy and Clinical Immunology, Volume 138, Issue 4, 2016, 1122–1130 http://dx.doi.org/10.1016/j.jaci.2016.03.041
Distinct gut microbiome composition associated with milk allergy resolution. Taxa within Clostridia and Firmicutes were enriched in children sampled at age 3 to 6 months with milk allergy resolution versus milk allergy persistence by age 8 years.

Supinda Bunyavanich, Nan Shen, Alexander Grishin, Robert Wood, Wesley Burks, Peter Dawson, Stacie M. Jones, Donald Y.M. Leung, Hugh Sampson, Scott Sicherer, Jose C. Clemente

Early-life gut microbiome composition and milk allergy resolution

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http://dx.doi.org/10.1016/j.jaci.2016.03.041
Dysbiosis in non-communicable diseases

Definitions

• **Prebiotics**- “A dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” (ISAAP 2008)

Commonly used prebiotics in infant formula: galacto- and fructooligosaccharides and their combination 9:1
Prebiotics

• Oligosaccharides are the third largest fraction in human milk and confer many potential benefits in breastfed infants
• Current commercially available prebiotics are less complex
• Prebiotics have the potential to promote gut colonization with bifidobacteria; some studies have demonstrated immune-stimulating effects
• Increases the production of short-chain fatty acids, with nutritive and anti-inflammatory effects

Definitions

- **Probiotics** “Live microorganisms, which when administered in adequate amounts confer a health benefit on the host” (FAO/WHO 2002)

  Commonly used probiotics are strains of bifidobacteria and lactobacilli
a) Block pathogen entry  
b) Create a mucus barrier  
c) Maintain intestinal integrity  
d) Produce antimicrobial factors  
e) Stimulate innate immune system via dendritic cells  
f) Stimulate/dampen innate immune response  

**Probiotics**—“live microorganisms which, when administered in adequate amounts confer a health benefit on the host”

WHO/FAO

Synbiotics

- Synbiotics - combination of pre- and probiotics
- Anticipated to have more global effects on colonization
- Meta-analysis eczema management (six studies, 369 children): SCORAD -6.56 (95% CI -11.43 to -1.68; P=0.008)
- “the effect most evident in children >1 yr and if the synbiotic contained a mix of bacterial strains”

Current recommendations

International expert bodies do not generally recommend pre- pro- or synbiotics for management or prevention of allergic disease.

Probiotics: “there is a likely net benefit from using probiotics resulting primarily from prevention of eczema” in infants at high risk of allergic disease.  
*WAO guideline panel 2015*

Prebiotics: “suggests using prebiotic supplementation in not-exclusively breastfed infants and not using prebiotic supplementation in exclusively breastfed infants” for allergy prevention.  
*WAO guideline panel 2016*

Recommendations conditional, based on very low certainty of the evidence.  

**Significant heterogeneity between studies**
• The gut microbiota develops to a complex and diverse ecosystem during the first years of life

• Aberrant intestinal colonization and reduced biodiversity have been associated with allergic diseases

• Pre- pro- and synbiotics have the potential to promote healthy gut colonization and immune maturation in allergic disease

• To date, significant heterogeneity between conducted studies precludes general recommendation on their use
Aim: Clinical study to assess the effect of an Amino Acid based Formula (AAF) with a synbiotic blend on gut microbiota and clinical symptoms in suspected GI Non-IgE mediated CMA

- Control product: Commercially available AAF
- Test product: AAF + synbiotics*; mixture of short and longchain fructooligosaccharides, *Bifidobacterium breve* M-16V

Studies suggest eHF + probiotics and/or prebiotics may have beneficial effect in allergy management (*Berni Canani 2012, Gruber 2010, Van de Aa 2010 & 2011*)

Very limited data regarding Non-IgE mediated allergic infants receiving AAF

*Michaelis et al. 2016*
## Previous studies including the specific synbiotic ingredients

Two double-blind randomized controlled clinical trials evaluated safety and growth (AAF vs AAF with synbiotics) and hypoallergenicity

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>Study length</th>
<th>Conclusions AAF with synbiotics</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy infants</td>
<td>115</td>
<td>16 weeks</td>
<td>- Safe&lt;br&gt;- Adequate growth in healthy infants</td>
<td>Harvey et al. 2014</td>
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<td>full term (0 - 15 days)</td>
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<tr>
<td>CMA infants</td>
<td>110</td>
<td>16 weeks</td>
<td>- Safe&lt;br&gt;- Adequate growth in CMA infants&lt;br&gt;- Suitable for management of CMA&lt;br&gt;- Exploratory outcome suggests less reported infections, less antibiotics</td>
<td>Burks et al. 2015</td>
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<tr>
<td>(0-8 months) confirmed IgE and/or non-IgE mediated</td>
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<tr>
<td>CMA infants/children</td>
<td>30</td>
<td>7 days</td>
<td>- Hypoallergenic (no allergic reaction to AAF with specific synbiotic ingredients in 30 CMA infants)</td>
<td>Harvey et al. 2014</td>
</tr>
<tr>
<td>(0-3 years) confirmed IgE with DBPCFC</td>
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ASSIGN – CLINICAL study

Methods:
• DB-RCT, 68 non-IgE mediated CMA infants (0-13 months) 8 wks intervention

Safety:
• Adverse events, medication use, growth

Primary parameters:
• Faecal bifidobacteria and *E. rectale* / *C. coccoides* group (FISH)

Secondary parameters:
• Stool characteristics, gut immune health markers

Exploratory parameters:
• Sequencing of faecal bacteria, clinical symptoms (SCORAD + parent reported, follow-up at 12 & 26 wks

Healthy breastfed group

Non-IgE CMA infants group

n=68

n=34

n=34

Test product for 8 weeks

Control product for 8 weeks

Test product optional

Control product optional

T8wks

T12wks

T26wks

Confidential
Development of core microbiota – A gradual transition from infant to adult-like

Cheng et al., ISME J. 2016;10(4):1002-14
### Demographics - Intention to treat

<table>
<thead>
<tr>
<th></th>
<th>Test (N = 35)</th>
<th>Control (N = 36)</th>
<th>Total CMA (N = 71)</th>
<th>Healthy subjects (N = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at baseline (months)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mean (SD)</td>
<td>5.67 (3.24)</td>
<td>6.33 (2.71)</td>
<td>6.00 (2.08)</td>
<td>7.84 (3.25)</td>
</tr>
<tr>
<td>Min - Max</td>
<td>1.8 - 12.8</td>
<td>1.2 - 11.6</td>
<td>1.2 - 12.8</td>
<td>2.6 - 14.2</td>
</tr>
<tr>
<td><strong>Sex (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>28.6</td>
<td>25.0</td>
<td>26.8</td>
<td>45.1</td>
</tr>
<tr>
<td>Male</td>
<td>71.4</td>
<td>75.0</td>
<td>73.2</td>
<td>54.9</td>
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<tr>
<td><strong>Race (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>5.7</td>
<td>2.8</td>
<td>4.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Black</td>
<td>2.9</td>
<td>0.0</td>
<td>1.4</td>
<td>0.0</td>
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<tr>
<td>Caucasian / White</td>
<td>88.6</td>
<td>88.9</td>
<td>88.7</td>
<td>92.2</td>
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<tr>
<td>Combination Of Above / Other</td>
<td>2.9</td>
<td>8.3</td>
<td>5.6</td>
<td>7.8</td>
</tr>
<tr>
<td><strong>Mode of delivery (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caesarean section</td>
<td>20.0</td>
<td>41.7</td>
<td>31.0</td>
<td>13.7</td>
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<tr>
<td>Vaginal</td>
<td>80.0</td>
<td>58.3</td>
<td>69.0</td>
<td>86.3</td>
</tr>
<tr>
<td><strong>Country of residence (%)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Belgium</td>
<td>17.1</td>
<td>13.9</td>
<td>15.5</td>
<td>0.0%</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>60.0</td>
<td>69.4</td>
<td>64.8</td>
<td>29.4</td>
</tr>
<tr>
<td>Italy</td>
<td>17.1</td>
<td>13.9</td>
<td>15.5</td>
<td>11.8</td>
</tr>
<tr>
<td>Sweden</td>
<td>5.7</td>
<td>2.8</td>
<td>4.2</td>
<td>58.8</td>
</tr>
</tbody>
</table>

N is number of subjects. Denominator for % is number of subjects in treatment group with non-missing data.

**PI ASSIGN: Dr. Louise Michaelis**

**Consultant Paediatrician in Immunology and Allergy**

**Great North Children’s Hospital, UK**
Primary outcomes in intention to treat analysis (ITT)
Increased level of – infant-like – Bifidobacteria

Statistics are based on ANCOVA comparing Test vs Control with Week 8 values as outcome, stratification factor (skin or gastrointestinal symptoms) and imputed baseline values as covariate and treatment as fixed effect.

PI ASSIGN: Dr. Louise Michaelis Great North Children’s Hospital, UK
Decreased level of – adult-like – E. Rectale / C. coccoides group

Statistics are based on ANCOVA comparing Test vs Control with Week 8 values as outcome, stratification factor (skin or gastrointestinal symptoms) and imputed baseline values as covariate and treatment as fixed effect.

PI ASSIGN: Dr. Louise Michaelis Great North Children’s Hospital, UK
ASSIGN – Conclusions

• The investigated AAF with synbiotics is safe and suitable for dietary management of infants with suspected Non-IgE mediated CMA

• Primary outcome met: Eight weeks use of AAF with specific synbiotics results in:
  ✓ Increase of infant like bifidobacteria
  ✓ Decrease of adult like *E. rectale/*C. coccoides group
  ✓ Bifidobacteria and *E. rectale/*C. coccoides levels in the test group are close to levels seen in age matched healthy subjects

• Further analysis ongoing for wk12 and wk26 clinical and fecal data; Wk 8 in depth microbiota (by sequencing method)
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  Heidi Sonnemans
  Rob Slump

Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands

Prof Jan Knol,
Harm Wopereis
Umeå university
A/Prof Patrik Rydén
Linda Vidman

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