Role of the Gut Microbiota in Early Life Immune Development and Oral Tolerance Induction

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• Immune mechanisms of oral tolerance induction

• Role of the intestinal microbiota in
  ✷ Immune development
  ✷ Oral tolerance
  ✷ Food sensitisation
Diet – Microbiota – Immune System Triad

Diet

Microbiota

Immune System

Homeostasis
Oral Tolerance

• First described by Wells and Osborne\(^1\) in 1911 ..... Guinea pigs did not develop anaphylaxis to ingested antigen

• Tolerance is an active immune response \(^2,3\)

Antigen Sampling

- Luminal antigens must be presented to immune cells for active induction of oral tolerance

- Antigen sampling can take place without disruption of the epithelial barrier via a number of pathways
  - M cell transcytosis
  - Goblet cell-associated passages
  - CX3CR1 dendritic cells
  - Paracellular leak

- These distinct pathways may preferentially sample antigens with different characteristics and selectively direct immune responses towards either inflammation or tolerance.
Oral Tolerance Induction Requires the Presence of Intestinal Microbiota
Intestinal Microbiota and Immune Development

• Mice bred in germ free conditions have abnormal Gut Associated Lymphoid Tissues (GALT)
  – Small underdeveloped Peyer’s Patches that lack germinal centres
  – Fewer IgA plasma cells and CD4+ T cells in the lamina propria
  – Fewer IEL with reduced cytolytic activity
  – These abnormalities can be corrected by seeding the intestine with *Bacteroides fragilis*

• Mice bred in germ free conditions have failure of oral tolerance induction and persistent Th2 responses
  – This can be corrected if reconstitute microbiota with *B fragilis*
  – But… ONLY if this occurs in the neonatal period

Intestinal Microbiota and Foxp3⁺ iTreg

- Germ free mice (that lack intestinal microbiota)¹⁻³
  - Reduced numbers of FoxP3⁺ iTreg in MLN +/- PP
  - iTreg have impaired suppressor function¹,² and reduced IL10 and TGFβ production²
  - Reduced production of IL12, IFNγ, IL10; and increased production of IL4³

Absence of Intestinal Microbiota Leads to Immune Dysregulation

- Germ-free mice have *elevated serum IgE* that develops in early life.

- *Immunoregulatory signals from microbiota are required to control basal IgE levels.*

Elevated IgE levels are not caused by food antigens

Cahenzli et al. Cell Host Microbe 2013
Elevated IgE levels in germ-free mice lead to increased mast-cell-surface-bound IgE and **exaggerated food anaphylaxis**.

Appropriate intestinal microbial stimuli during early life are critical for inducing an immunoregulatory network that protects against induction of IgE at mucosal sites.

Mice were sensitized s.c. with Alum alone (open symbols) or ALUM + OVA (closed symbols) and challenged with 50 mg OVA orally (n = 6–9 per group).

Cahenzli et al. Cell Host Microbe 2013
Clostridia and Epithelial Barrier Function

- Clostridia, a class of anaerobic Firmicutes that reside in close proximity to the colonic epithelium, can modulate sensitisation to food allergens and the allergic response.
- Colonisation of germ free mice with Clostridia group can prevent sensitization to food allergens and reduce severity of anaphylaxis.
Clostridia Species Prevent Food Allergen Sensitisation and Protect Against Food Anaphylaxis

- GF mice sensitised with PN/CT have increased levels of PN-specific IgE and more severe anaphylaxis compared to SPF mice.

- Colonisation of GF mice with a consortium of Clostridia (but not B uniformis) protected against sensitization to PN/CT and against anaphylaxis

Stefka et al. PNAS 2014;111:13145–13150
Clostridia Species Prevent Passage of Food Allergen into the Circulation

Reduced passage of antigen mediated by IL22 and increased goblet cell number / function

Stefka et al. PNAS 2014;111:13145–13150
Clostridia Species Induces iTreg and IgA (at 14 days)

Stefka et al. PNAS 2014;111:13145–13150
Are alterations in the gut microbiota associated with loss of oral tolerance in humans?
Altered Intestinal Microbiota in Infants with Eczema

- Children with eczema have altered microbiota \(^1-^3\)
  - lower counts of Bifidobacteria and Lactobacilli
  - higher counts of \textit{S} \textit{aureus}, \textit{C} \textit{difficile} and \textit{E} \textit{coli}

- These differences \textit{precede} the onset of allergic disease \(^2,^4,^5\)
  - lower counts bifidobacteria and enterococci at 1 mos
  - lower counts of bifidobacteria and bacteroides at 12 mos
  - higher counts of \textit{C} \textit{difficile} and \textit{E} \textit{Coli} at 3 weeks and 3 mos
  - more often colonised with \textit{S} \textit{aureus} at 6 mos

The composition of bifidobacterium flora is also different in children with allergic disease

- Specific strain differences vary in different geographic locations

Scandinavia

- allergic subjects have predominance of *B adolescentis* (adult-type) \(^1\)
- healthy infants have predominance of *B bifidum and B catenulatum / pseudocatenulatum* \(^1,2\)
- reduced adhesion to human intestinal mucus \(^3\)
- induce less IL10 production in vitro \(^4\)

Japan \(^5,6\) and New Zealand \(^7\)

- Allergic subjects higher levels of *B bifidum* (Japan) and *B catenulatum* (Japan, NZ)

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Importance of Microbial Diversity in Early Life

Ismail et al. Pediatr Allergy Immunol 2012;23:674-81

Abrahamsson et al. J Allergy Clin Immunology 2012;129:434
Diet—1 medicine mode

Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation

Kei E Fujimura¹, Alexandra R Sitarik², Suzanne Havstad², Din L Lin¹, Sophia Levan¹, Douglas Fadros⁹, Ariane R Panzer¹, Brandon LaMere¹, Elze Rackaityte¹, Nicholas W Lukacs³, Ganesa Wegienka², Homer A Boushey⁴, Dennis R Ownby⁵, Edward M Zoratti⁶, Albert M Levin², Christine C Johnson²,⁷ & Susan V Lynch¹,⁷

The n innate immunity

REVIEW

Microbiome-wide association studies link dynamic microbial consortia to disease

Jack A. Gilbert¹, Robert A. Quinn² ⁴, Justine Debelius⁵, Zhenjiang Z. Xu⁵, James Morton⁵, Neha Garg² ² ³, Janet K. Jansson⁷, Pieter C. Dorrestein² ² ³ & Rob Knight⁴ ⁶
Altered Fecal Bacteria in Infants with CMPA

<table>
<thead>
<tr>
<th>Counts</th>
<th>Group</th>
<th>Baseline</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median (95% CI)</td>
<td>Median (95% CI)</td>
</tr>
<tr>
<td>Aerobes</td>
<td>CMPA</td>
<td>9.16 (8.81–9.61)</td>
<td>9.05 (8.56–9.21)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>9.18 (8.87–9.47)</td>
<td>8.90 (8.19–9.68)</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>CMPA</td>
<td>10.42 (10.19–10.65)†</td>
<td>10.41 (10.10–10.67)*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10.18 (9.89–10.41)</td>
<td>10.23 (9.98–10.37)</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>CMPA</td>
<td>8.79 (8.41–9.17)</td>
<td>8.55 (7.99–8.67)§</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8.78 (8.38–9.12)</td>
<td>8.54 (7.76–8.99)</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>CMPA</td>
<td>7.23 (4.71–7.45)</td>
<td>9.34 (8.47–9.69)¶</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.29 (6.12–7.82)</td>
<td>7.00 (6.30–8.32)</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>CMPA</td>
<td>9.33 (8.35–10.08)</td>
<td>7.91 (5.76–9.26)¶</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>9.43 (7.82–9.91)</td>
<td>9.35 (7.85–9.72)</td>
</tr>
<tr>
<td>Clostridia</td>
<td>CMPA</td>
<td>5.24 (4.35–6.15)</td>
<td>5.36 (4.51–6.35)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.35 (4.99–6.39)</td>
<td>5.48 (5.11–6.06)</td>
</tr>
<tr>
<td>Yeast</td>
<td>CMPA</td>
<td>3.84 (3.49–5.00)‡</td>
<td>4.54 (3.93–5.76)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.03 (4.07–5.69)</td>
<td>4.61 (4.11–5.81)</td>
</tr>
<tr>
<td>Total count</td>
<td>CMPA</td>
<td>10.48 (10.26–10.72)*</td>
<td>10.44 (10.15–10.67)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10.32 (10.04–10.38)</td>
<td>10.38 (10.13–10.58)</td>
</tr>
</tbody>
</table>

Median (95% confidence interval) of logarithmic colony forming units (CFU).
CMPA vs. control: *p < 0.05, †p < 0.01, ‡p < 0.001; baseline vs. 6 months: §p < 0.01, ¶p < 0.001.

Thompson-Chagoyan et al. Paediatr Allergy Immunol 2010
• 45 food sensitised and healthy children (age 6-23 mos)
  – 454 high-throughput 16S rRNA pyrosequencing

• Food sensitised children
  – Lower alpha diversity (Shannon index)
  – Lower diversity of the total microbiota (1.46 vs. 1.70, p = 0.01) and the phylum Bacteroidetes (1.51 vs. 1.73, p = 0.02)
  – Differences were statistically significant at the phylum, class, and order levels (p < 0.05).
  – Significantly lower overall gut microbiota richness (mean Chao1 richness estimator: 25.85 vs. 27.92, respectively, p = 0.04)

Alterations in the gut microbiota of children with food sensitization in early life

Phylum Level

Food sensitization (n = 23)
- Firmicutes
- Bacteroidetes
- Proteobacteria
- Actinobacteria
- others

Healthy control (n = 22)
- Firmicutes
- Bacteroidetes
- Proteobacteria
- Actinobacteria
- others

Class and Order Level

Food sensitization (n = 23)
- Bacteroidales
- Clostridiales
- Selenomonadales
- Enterobacteriales
- Lactobacillales
- Erysipelotrichales
- others

Healthy control (n = 22)
- Bacteroidales
- Clostridiales
- Selenomonadales
- Enterobacteriales
- Lactobacillales
- Erysipelotrichales
- others

**Increased abundance** of Firmicutes (phylum), Clostridia and Alphaproteobacteria (class), Clostridiales (order), and *Clostridium IV* and *Subdoligranulum* (genus) together with **decreased abundance** of Bacteroidetes (phylum), Bacteroidia (class), Bacteroidales (order), Bacteroidaceae (family), *Bacteroides* and *Veillonella* (genus) could be used to discriminate food sensitization.
Can alterations in the gut microbiota cause food allergy?
Mice susceptible to food allergy have altered microbiota

**IL4raF709 mice**
- Carry a gain of function mutation in IL4R α chain
- Susceptibility to experimentally induced food allergy

Noval Rivas M et al. JACI 2013;131:201-12
Intestinal microbiota from sensitised Il4raF709 mice transmits susceptibility to experimentally induced food allergy

Noval Rivas M et al. JACI 2013;131:201-12
Can we modulate the intestinal microbiota to support oral tolerance?
Establishment of the Intestinal Microbiota

• Gut microbiota evolves rapidly from birth ➔ Relatively stable by 3 years
  – First bacterial communities (seeding bacteria) are acquired from the mother and/or the environment
  – Diversity increases over the first 3 years in response to weaning diet / solids

• Microbiota composition is influenced by early life exposures
  – Mode of delivery (cesarean vs vaginal)
  – Maternal microbiota (stress/diet during pregnancy)
  – Infant diet
  – Sanitation and level of cleanliness
  – Antibiotic therapy

• Stable microbiota is largely established by age 3 years and remains resilient to acute environmental changes
  – Compositional homeostasis
Summary

- The intestinal microbiota plays a critical role in immune development and establishment of oral tolerance.
- Alterations in the intestinal microbiota in early life → increased risk of eczema (and other allergic conditions).
- In mouse models, transfer of disease associated microbial signatures can confer susceptibility to food allergy.
- Modification of the intestinal microbiota in early life may offer a novel approach to supporting healthy immune homeostasis.