Biotics in neonatal development

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Microbial colonization of the infant GI tract

"The microbial colonization of the infant GI tract is a remarkable episode in the human lifecycle"

Microbial colonization of the infant GI tract

"The microbes that colonize mucosal tissues after birth play a pivotal role in shaping the development of the host immune system"

Ash & Mueller, Science 2016;352:531
The intestinal tract of the infant

• Is not mature at the time of birth
• Is the largest surface area that is exposed to the environment, especially a vast array of luminal microbes and antigens
• The mucosal immune system represents about 80% of the infant's immune system
• The development of the infant's immune system is closely related to the intestinal microbiota
Mode of delivery determines ... early intestinal colonization
Brumbaugh et al., JPGN 2016;63:320

"There is accumulating evidence that intestinal bacteria play an important role in the postnatal development of the immune system" Björksten 2004
"The immune system undergoes major development during infancy, and the development is highly related to the microbes that colonize the intestinal tract"

How does the infant acquire his/her microbiome?

• Before birth the gut is sterile
• Colonization due to exposure to vaginal microflora
• Supplemented by bacteria contained in breast milk and skin bacteria
<table>
<thead>
<tr>
<th></th>
<th>Exposure at birth</th>
<th>Seeding of GI tract</th>
<th>Immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vaginal delivery</strong></td>
<td>vaginal microflora</td>
<td>normal</td>
<td>normal development</td>
</tr>
<tr>
<td><strong>Cesarean delivery</strong></td>
<td>no exposure to vaginal microflora</td>
<td>abnormal</td>
<td>abnormal development</td>
</tr>
</tbody>
</table>
Cesarean delivery

- Lack of exposure to vaginal microflora
- Abnormal seeding of GI tract with environmental flora, food flora
- Difference in microbiome detectable up to one year
- Abnormal development of immunity
Mode of delivery determines ... early intestinal colonization

Brumbaugh et al., JPGN 2016;63:320

"CS infants exhibited different microbiota in the oral inoculum, a chaotic pattern of bacterial succession, and a persistent deficit of intestinal Bacteriodetes"
Development of the human infant intestinal microbiota

"The composition and temporal patterns of the microbial communities varied widely from baby to baby"

"Despite considerable temporal variation, the distinct features of each baby were recognizable for weeks and months"

"By the end of the first year of life, the microbial systems in each baby had converged to a profile characteristic of the adult GI tract"
Cesarean delivery

Summary

Modification of long-term risk of several major diseases provides irrefutable evidence of modification of the immune system
Cesarean delivery-associated childhood diseases

Neu & Rushing, Clin Perinatol 2011; 36:321-331

<table>
<thead>
<tr>
<th>Disease</th>
<th>Odds Ratio</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic rhinitis</td>
<td>1.37</td>
<td>(1.14-1.63)</td>
</tr>
<tr>
<td>Asthma</td>
<td>1.24</td>
<td>(1.01-1.53)</td>
</tr>
<tr>
<td>Celiac disease</td>
<td>1.80</td>
<td>(1.13-2.88)</td>
</tr>
<tr>
<td>Diabetes type 1</td>
<td>1.19</td>
<td>(1.04-1.36)</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>1.31</td>
<td>(1.24-1.38)</td>
</tr>
<tr>
<td>Gastroenteritis and asthma</td>
<td>1.74</td>
<td>(1.36-2.23)</td>
</tr>
</tbody>
</table>
Cesarean delivery and risk of obesity in offspring
(Blustein et al., Int J Obesity 2013;37:900-906)

Avon Longitudinal Study
9.06% born by cesarean

Results: Increased adiposity beginning at age 6 weeks (+0.11SD units, p=0.005) through age 15 years (+0.10 SD units, p=0.042)
Age 11 yrs: OR 1.83; higher with obese than with non-obese mothers
What is the microbiome of the infant?

- There is not a "normal" microbiome
- But we can describe the microbiome of a breastfed full-term infant who was born vaginally
- It consists nearly exclusively of Lactobacilli and Bifidobacteria
Microbiota of the breastfed infant

Lactobacillus

Bifidobacterium
Microbiota of the infant

The good guys: Lactobacilli and Bifidobacteria (fucose-consuming bacteria)

The not so good guys: All others (E. coli, Clostridia) including pathogens
Microbiota of the breastfed infant

- The infant receives a constant supply of gram+ bacteria (milk, skin)
- Human milk provides oligosaccharides that favor bifidobacteria and lactobacilli
- Human milk contains immunoglobulin A
- Together they suppress undesirable bacteria and pathogens
Microbiota of the breastfed infant

- Human milk contains live bacteria
- Human milk contains oligosaccharides that favor good bacteria and inhibit bad bacteria
- Development of immunity differs
Breastfed infant
Formula-fed infant
Human milk oligosaccharides (1)

- About N=300
- Fucosylated (70-80%) and sialylated
- Homologs of blood type antigens
- 2’-fucosyllactose (2’-FL) dominant (“secretor”)
- 20-25% of mothers lack 2’-FL in their milk (“non-secretor”)
- Composition and concentration change during lactation
Human milk oligosaccharides vs prebiotics added to formulas

Monosaccharide key:
- glucose
- galactose
- N-acetylglucosamine
- fucose
- N-acetyleneuraminic acid
- fructose

Ninonuevo & Bode, Pediatr Res 2008;64:8
Long-term benefits of breastfeeding

• Lower risk of obesity
• Lower risk of cardiovascular disease and metabolic syndrome
• Lower risk of leukemia
Prebiotics in infants

- For practical reasons mostly limited to provision of galacto-oligosaccharides and fructo-oligosaccharides
- Addition to formulas mostly produces softer stool
L Bode, Nutr Rev 2009;67:S183-S191
Prebiotics in infants
Rationale: Imitate effects of breast milk oligosaccharides

• Several studies show that addition of prebiotics leads to softer stools

• Two studies showed reduced early atopic dermatitis with formula with neutral oligosaccharides and pectin-derived acidic oligosaccharides (Grüber et al., J Allergy Clin Immunol 2010)
Infants fed prebiotics had reduced atopic dermatitis in the first 6 months of life

Stool Data

- **Microbiota:**
  - Increase of bifidobacteria (but not of lactobacilli) at 3 and 6 months
- **Stool frequency:**
  - Increase at 3 and 6 months
- **Stool consistency:**
  - Decrease at 3 and 6 months

Grüber et al., J Allergy Clin Immunol 2010
Probiotics in infants: Rationale for use

• Increase the concentration of Bifidobacteria and/or Lactobacilli to
  – help restore the balance of the GI flora when diet is sterile infant formula
  – Compete with invasive bacteria for receptors to enhance gut barrier function
  – Help increase levels of immunoglobulins such as secretory IgA
Probiotics in infants

- Can be given as such or mixed in formula
- Most widely used are B. bifidum, B. infantis and L Reuteri
- Effects: decreased diarrhea, softer stools, less constipation, less colic
- No adverse effects
Use of antibiotics

- Use of antibiotics has profound effects on the microbiome
- Recovery of a normal microbiome can take weeks and months
Colonocyte metabolism determines gut health

P T Cani, Science 2017, 357: 548-49
Preterm Newborn
Microbiota of the preterm infant

- Birth almost always by cesarean method
- Universally antibiotics at least for a few days
- Abnormal and sparse microbiota
- Susceptibility to necrotizing enterocolitis
The immature gut (1)

- Diminished cell mass, enzyme activity
- Increased permeability
- Disordered, immature motility
- Susceptibility to NEC
- Absent or abnormal microbiota
The immature gut (2)

- Maturation occurs rapidly (usually <2 weeks)
- Maturation depends on stimulation by nutrients ("trophic feeds")
- Nutrients need only to reach stomach
- Maturation of motility is used as an indicator of maturation, but not all functions mature at the same rate (e.g., susceptibility to NEC)
Premature infants
Objectives of use of microbiota

- Restore or establish normal microbiota
- Exert beneficial effects on gut mucosa

Clinical effect(s):
- Protection against NEC
- Facilitate early feeding advancement
7 RCT analyzed (n=1393 infants).

Probiotics: Singly or in combinations of 2 or 3
Start of administration: First feed or day 1
End of administration: 21 days, 28 days, 36 weeks, discharge
Once or twice daily
Doses differed between studies
Outcomes: NEC (n=3), gut colonization, gut function
Deshpande G, Rao S and Patole S:

Updated Meta-analysis of Probiotics for Preventing Necrotizing Enterocolitis in Preterm Neonates

Pediatrics 2010; 125:921-930

11 RCT analyzed, N=2176 infants
(4 RCT had NEC or NEC and death as primary outcomes)
<table>
<thead>
<tr>
<th>Probiotics used</th>
<th># Studies</th>
</tr>
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<tbody>
<tr>
<td>Bifidobacterium breve</td>
<td>1</td>
</tr>
<tr>
<td>Bifidobacterium lactis</td>
<td>3</td>
</tr>
<tr>
<td>Lactobacillus GG</td>
<td>2</td>
</tr>
<tr>
<td>Saccharomyces boulardii</td>
<td>1</td>
</tr>
<tr>
<td>Bifidobacterium infantis</td>
<td>3</td>
</tr>
<tr>
<td>Bifidobacterium bifidum</td>
<td>3</td>
</tr>
<tr>
<td>Streptococcus thermophilus</td>
<td>1</td>
</tr>
<tr>
<td>Lactobacillus acidophilus</td>
<td>2</td>
</tr>
<tr>
<td>Lactobacillus casei</td>
<td>1</td>
</tr>
<tr>
<td>Bifidobacterium longum</td>
<td>1</td>
</tr>
</tbody>
</table>
Effect of probiotics on NEC

Review: Probiotics for prevention of necrotizing enterocolitis
Comparison: 01 NEC
Outcome: 01 Definite NEC

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Probiotic</th>
<th>no probiotic</th>
<th>RR (fixed)</th>
<th>Weight</th>
<th>RR (fixed)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n/N</td>
<td>n/N</td>
<td>95% CI</td>
<td>%</td>
<td>95% CI</td>
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<tr>
<td>Kitajima 1997</td>
<td>0/45</td>
<td>0/46</td>
<td>Not estimable</td>
<td></td>
<td></td>
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<tr>
<td>Dani 2002</td>
<td>4/295</td>
<td>8/290</td>
<td>11.15</td>
<td>0.49</td>
<td>[0.15, 1.61]</td>
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<tr>
<td>Costalos 2003</td>
<td>5/51</td>
<td>6/36</td>
<td>9.72</td>
<td>0.59</td>
<td>[0.19, 1.78]</td>
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<tr>
<td>Bin Nun 2005</td>
<td>1/72</td>
<td>10/73</td>
<td>13.73</td>
<td>0.10</td>
<td>[0.01, 0.77]</td>
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<tr>
<td>Lin 2005</td>
<td>2/180</td>
<td>10/187</td>
<td>13.56</td>
<td>0.21</td>
<td>[0.05, 0.94]</td>
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<tr>
<td>Manzoni 2006</td>
<td>1/39</td>
<td>3/41</td>
<td>4.04</td>
<td>0.35</td>
<td>[0.04, 3.23]</td>
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<tr>
<td>Mohan 2006</td>
<td>2/21</td>
<td>1/17</td>
<td>1.53</td>
<td>1.62</td>
<td>[0.16, 16.37]</td>
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<tr>
<td>Stratiki 2007</td>
<td>0/38</td>
<td>3/31</td>
<td>5.31</td>
<td>0.12</td>
<td>[0.01, 2.19]</td>
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<td>Lin 2008</td>
<td>4/217</td>
<td>14/217</td>
<td>19.35</td>
<td>0.29</td>
<td>[0.10, 0.85]</td>
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<tr>
<td>Samanta 2008</td>
<td>5/91</td>
<td>15/95</td>
<td>20.29</td>
<td>0.35</td>
<td>[0.13, 0.92]</td>
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<tr>
<td>Rouge 2009</td>
<td>2/45</td>
<td>1/49</td>
<td>1.32</td>
<td>2.18</td>
<td>[0.20, 23.21]</td>
</tr>
</tbody>
</table>

Total (95% CI): 1094 | 1082 | 100.00 | 0.35 | [0.23, 0.55] |

Total events: 26 (Probiotic), 71 (no probiotic)
Test for heterogeneity: Chi² = 7.66, df = 9 (P = 0.57), I² = 0%
Test for overall effect: Z = 4.64 (P < 0.00001)

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Effect of probiotics on all-cause mortality

<table>
<thead>
<tr>
<th>Study</th>
<th>Probiotic</th>
<th>No probiotic</th>
<th>RR (fixed)</th>
<th>Weight</th>
<th>RR (fixed)</th>
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<tr>
<td>Kitajima 1997</td>
<td>0/45</td>
<td>2/46</td>
<td>2.85</td>
<td>0.20</td>
<td>[0.01, 4.14]</td>
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<tr>
<td>Dani 2002</td>
<td>12/295</td>
<td>22/290</td>
<td>25.59</td>
<td>0.54</td>
<td>[0.27, 1.06]</td>
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<tr>
<td>Bin Nun 2005</td>
<td>3/72</td>
<td>9/73</td>
<td>10.31</td>
<td>0.34</td>
<td>[0.10, 1.20]</td>
</tr>
<tr>
<td>Lin 2005</td>
<td>7/180</td>
<td>20/187</td>
<td>22.63</td>
<td>0.36</td>
<td>[0.16, 0.84]</td>
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<tr>
<td>Manzoni 2006</td>
<td>5/39</td>
<td>6/41</td>
<td>6.75</td>
<td>0.88</td>
<td>[0.29, 2.64]</td>
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<tr>
<td>Stratiki 2007</td>
<td>1/38</td>
<td>1/31</td>
<td>1.27</td>
<td>0.82</td>
<td>[0.05, 12.52]</td>
</tr>
<tr>
<td>Lin 2008</td>
<td>2/217</td>
<td>9/217</td>
<td>10.38</td>
<td>0.22</td>
<td>[0.05, 1.02]</td>
</tr>
<tr>
<td>Samanta 2008</td>
<td>4/91</td>
<td>14/95</td>
<td>15.80</td>
<td>0.30</td>
<td>[0.10, 0.87]</td>
</tr>
<tr>
<td>Rouge 2009</td>
<td>2/45</td>
<td>4/49</td>
<td>4.42</td>
<td>0.54</td>
<td>[0.10, 2.83]</td>
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<tr>
<td>Total (95% CI)</td>
<td>1022</td>
<td>1029</td>
<td>100.00</td>
<td>0.42</td>
<td>[0.29, 0.62]</td>
</tr>
</tbody>
</table>

Total events: 36 (Probiotic), 87 (No probiotic)

Test for heterogeneity: Chi² = 4.01, df = 8 (P = 0.86), I² = 0%

Test for overall effect: Z = 4.49 (P < 0.00001)


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Probiotics in premature infants

Benefits

• Restore or establish normal microbiota
• Exert beneficial effects on gut mucosa

Clinical effect(s): Protect against NEC
Facilitate early feeding advancement
Probiotics in premature infants
Practice at UIHC

All premature infants receive probiotics providing: Lactobacillus rhamnosus GG
Bifidobacterium breve
Bifidobacterium bifidum
Bifidobacterium infantis
Bifidobacterium longum

2 x 10^9 CFU per dose