Human Milk Fortifier and the Preterm Infant Gut Microbiome

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The speaker has signed a disclosure form and indicated she has no significant financial interest or relationship with the companies or the manufacturer(s) of any commercial product and/or service that will be discussed as part of this presentation.

Session Summary

Preterm infants have special needs and vulnerabilities. Mothers' own milk may be uniquely designed for their own infants' needs and to reduce risks. Milk has a unique microbiome that functions as the pioneer foundation of an infant’s gut microbiome. This talk will describe our research program in human milk biology and the preterm infant gut microbiome.

Session Objectives

Upon completion of this presentation, the participant will be able to:

- define the composition of the normal infant gut microbiome;
- describe the role of the gut microbiome in health;
- identify key phyla and timing in the infant gut microbial succession;
- explain mechanisms that influence the normal infant gut microbiome;
- compare the term and preterm infant gut microbial development;
- explain the role of human milk in microbial development;
- discuss the role of the milk microbiome;
- distinguish dysbiosis in the preterm infant gut;
- predict potential effects of preterm infant gut dysbiosis on long term health.

References


Milk and the gut microbiome with special emphasis on the Very Low Birth weight infants

Bacteria are Us

Bacteria have inhabited the earth for at least two and a half billion years. Our evolutionary ancestors arrived in a world dominated by microbes, and, as we evolved, so did they.

Genes

- We humans have ~23,000
- We share up to 20 million with our microbes

- 100 trillion beneficial microorganisms—bacteria, fungi, and viruses—populate the body and are necessary for health
Commensals vs Pathogens

- The beneficial commensals must be recognized and tolerated by the immune system
- The virulent pathogens must be attacked by the immune system
- The immune system is shaped by early life exposures to microbial life

Early microbes provide the pioneer culture for the development of the commensal gut microbiome

Commensals

- Contain polysaccharide-digesting enzymes that are not present in the human genome
- Dietary polysaccharides are degraded in the gut by bacteria
- Commensals inhibit growth and penetration of pathogens
- Make vitamins
- Tolerize the immune system

Other Commensal Functions

- Direct contact of bacterial cells necessary for development, regulation and response of the immune system
- Bacteria produce key metabolites that cross into bloodstream
- Bacteria produce amino acids (e.g., tryptophan) that can affect levels of serotonin and other neurotransmitters
- Bacteria have different "metabolic rates" so some are more or less efficient … can result in obesity

Commensal growth inhibits ability of virulent pathogens to penetrate gut mucus and epithelium

At Birth

- Old idea was that infants were born sterile
  - Now known that meconium has a microbiota, placenta has a microbiota
  - Infant is born with a small maternally originated (largely vaginal) microbiota
within hours after birth the infant is heavily colonized

The first exposures

- Maternal vaginal, enteric and skin microbes as the infant is delivered vaginally, and immediately placed on the mother’s abdomen and allowed to latch
- And then: Breastmilk!

Acquisition of the earliest microbiome

Microbiology 101

- Phylum: Firmicutes
- Class: Bacilli
- Order: Lactobacillales
- Family: Lactobacillaceae
- Genus: *Lactobacillus* (*Gm+*, facultative anaerobic, rod shaped)
- Species: many hundreds

Milk has a microbiome: the pioneers

- *microbiome in human milk*, the first food introduced into the gastrointestinal tract, and which may orchestrate, program and time the future development of the communities of microbes living in the child’s gut.
- Signature gut microbiome develops early
Components of the milk microbiome

- $10^3$ to $10^4$ colony forming units in every ml of human milk (culturable)
- Most frequently cultured bacteria in human milk are: Staphylococcus, Streptococcus, Lactococcus, Weissella, Enterococcus, Propionibacterium, Lactobacillus, and Bifidobacterium.
- NGS has identified far more diversity

In the past....
"Do not use alcohol on the breast...clean with "soap" and water before feeding"

Breastmilk

- "lean"-promoting microbiota: Increased Bacterioides, decreased Firmicutes

Influences

- Little known about what influences milk microbiota
  - Specific microbial genera were reported to change over the time of lactation
  - Obese women had less diversity and a different microbiome compared to lean mothers
  - Microbiome changes over time and different dependent upon whether mother is exclusively or partially breastfeeding

Source of Microbes

- Most from maternal GI tract
- One idea is that dendritic cells sample, engulf, and transport bacteria and home to the lactating breast
- Another idea is that bacteria come from infant's mouth

Milk Biochemistry and the infant gut

- More gut Bifidobacteria 60-90% in breastfed; ~50% in Formula fed; FF also have higher Bacteroides, Clostridia, Enterococcus, Staphylococcus
- Rich diversity of HMOs...exceeding other species by up to 100%. Promote Bifidobacteria
- BF babies gut microbiota contain bacteria specialized to metabolize HMOs
Microbiome influences Gut Development

Gene networks differentially expressed in exfoliated epithelial cells from breast- and formula-fed infants. Gene expression was determined in exfoliated intestinal epithelial cells from 3-mo-old breast- and formula-fed infants.

Ecology of the Human Milk Microbiome and Infant saliva

Comparison of ecological influences in Kenya tribe compared to Tampa mothers

Human Microbiome
Microbiome Influences Phenotype

- Obesity
- Autoimmune disease
- Allergy
- ASD
- Depression
- Anxiety
- GI disease
- Heart disease
- Mercury toxicity

Disease | Relevant finding
---|---
Psoriasis | Increased ratio of Firmicutes to Actinobacteria
Reflux esophagitis | Esophageal microbiota dominated by gram-negative aerobes
Obesity | Gastric microbiota with low or absent H. pylori
Obesity | Reduced ratio of Bacteroidetes to Firmicutes
Childhood-onset asthma | Absent gastric Helicobacter pylori (especially cytotoxin-associated gene (cagA) genotype)
IBD (colitis) | Increased Enterobacteriaceae
Functional bowel diseases | Increased Veillonella and Lactobacillus
Colorectal cancer | Increased Fusobacterium spp.
Cardiovascular disease | Gut microbiota-dependent metabolism of phosphatidylcholine

The Gut Microbiome

Gut microbiome interacts with immune system

Pathogens and Leaky Gut Theory

- If dysbiosis, inflammation and leaky gut can occur
- Toxins, organism, undigested food, medications, metabolites, can leak out
- Immune response produces potential widespread effects...diabetes, asthma, lupus, multiple sclerosis, depression, anxiety, autism

Leaky Gut
Regulation of the microbiota-brain-gut axis is essential for maintaining homeostasis, including that of the CNS.

The germ free mouse

- A model for what happens when an organism does not have a gut microbiome

Germ free mouse incubators at NIH

Behavior in the germ free mouse

- Increased response to stress
- More daring
- Reduced anxiety
- Reduced non-spatial memory
- Altered monoamines
- Lack an ability to recognize other mice with whom they interact
- Altered neurotrophin levels

Microbiota can then be manipulated in germ free mice

- When colonization of the intestines of one strain of germ-free mice with bacteria taken from the intestines of another mouse strain: the recipient animals would take on aspects of the donor's personality. Naturally timid mice would become more exploratory, whereas more daring mice would become apprehensive and shy.
- These tendencies suggested that microbial interactions with the brain could induce anxiety and mood disorders.

The household microbiome (Dr. Jack Gilbert, Argonne National lab) \(\text{Science,}\ 2014\ \text{Aug 29;345(6200):1048-52}\)

The Home Microbiome Project followed seven families, which included 18 people, 3 dogs and 1 cat, over the course of 6 weeks. The participants in the study swabbed their hands, feet and noses daily to collect a sample of the microbial populations living in and on them. They also sampled surfaces in the house, including doorknobs, light switches, floors and countertops.
Home is where the microbes are

- They found that people substantially affected the microbial communities in a house—when three of the families moved, it took less than a day for the new house to look just like the old one, microbiologically speaking.

Behavior

- Microbiota NEED us to be social
- Gut-Brain axis involved in behavior

Microbiome and ASD

- Women who suffer from a high, prolonged fever during pregnancy are up to seven times more likely to have a child with autism.
- 40 to 90 percent of all children with autism suffer from gastrointestinal symptoms
- Dysbiosis has been noted in gut
- Neuroinflammation
- Study of vancomycin Rx reversing Sx

New meaning to “gut feelings”

Preterm Infant Gut Microbiome

- Very abnormal: low in anaerobes, sparse, staph, enterococci, enterobacter, yeasts
- Signature does not develop at same rate at term

HEALTH
- Normal range of social and feeding behaviors
- Normal gastrointestinal functions
- Normal gut permeability and gut motility
- Normal level of cytokines
- Normal microflora

AUTISM
- Altered range of social and feeding behaviors
- Altered function(s) of social and food-reward control(s)
- Altered gastrointestinal functions
- Altered gut permeability
- Altered gut motility
- Altered levels of cytokines
- Altered levels of brain neurotransmitters
- Altered biodiversity of gut microbiome; dysbiosis
Influences on VLBW Infants’ Gut Microbiota
- Caesarean section vs. vaginal birth
- Amount of human milk
- Antibiotics
- Infections
- Prenatal exposures
- Gestational age
- Genetics
- Invasive procedures
- Stress and trauma
- Separation from parents
- Where born

Normal Bacterial Succession in Infants
- Pioneer bacteria compete for substrate and adhesion sites (reduce high redox potential and allow anaerobes). Early on low diversity of facultative anaerobes (Enterobacteriacea (Proteobacteria) such as Shigella, Eschericia, Streptococcacea (Firmicutes)
- Within days, strict anaerobes flourish: Actinobacteria (eg; Bifidobacterium)

The signature microbiome (up to 3000 species)
- Most term infants achieve this around 2-3 years of age
- It remains one’s “signature” for life
- Influenced by food, household, diet, antibiotics and other exposures such as pets

The gut microbiome
- Enormous implications for health… and not just gastrointestinal health!
- One’s signature microbiome can ultimately affect how much a person weighs, how they behave, their development, and health in many different physiological systems

Sample
  - 80 VLBW infants followed for 6 weeks in the NICU
Stool collection

The Preterm Infant Microbiome: Biological, Behavioral and Health Outcomes at 2 and 4 years of Age

• R01NR015446

Specific Aim 1

To determine relationships between VLBW infant gut microbial succession and abundances (16S rRNA V3-V4 region amplified by Illumina MiSeq) while in the NICU with

- Human milk volumes and immunobiology
- Sickness
- Gestational age and weight
- Antibiotic exposure
- Fecal calprotectin
- Maternal factors

Sample

<table>
<thead>
<tr>
<th>Infant Characteristics</th>
<th>Means</th>
</tr>
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<tbody>
<tr>
<td>Birth Weight (Grams)</td>
<td>1081</td>
</tr>
<tr>
<td>Apgar at 1 minute</td>
<td>7</td>
</tr>
<tr>
<td>Apgar at 5 minutes</td>
<td>8</td>
</tr>
<tr>
<td>Gestational Age</td>
<td>28 weeks</td>
</tr>
<tr>
<td>C-section</td>
<td>76%</td>
</tr>
<tr>
<td>Males</td>
<td>54%</td>
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<tr>
<td>Antibiotics</td>
<td>88%</td>
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</table>

Perinatal Morbidity in the Sample

<table>
<thead>
<tr>
<th>Morbidity</th>
<th>Number of Infants (N=80)</th>
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<tbody>
<tr>
<td>PFO or PDA</td>
<td>23 (30%)</td>
</tr>
<tr>
<td>Other Cardiac problem</td>
<td>8 (10.5%)</td>
</tr>
<tr>
<td>CLD</td>
<td>4 (5.3%)</td>
</tr>
<tr>
<td>IUGR</td>
<td>11 (14.5%)</td>
</tr>
<tr>
<td>BOP</td>
<td>14 (18.4%)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>11 (14.5%)</td>
</tr>
<tr>
<td>IVH</td>
<td>9 (11.8%)</td>
</tr>
<tr>
<td>NEC</td>
<td>3 (3.9%)</td>
</tr>
<tr>
<td>Death</td>
<td>2 (2.6%)</td>
</tr>
</tbody>
</table>

PFO=patent foramen ovale  PDA=patent ductus arteriosus  CLD=chronic lung disease  IUGR=intrauterine growth restriction  BOP=intrapartum opaunum  IVH=intraventricular hemorrhage  NEC=necrotizing enterocolitis
Results

Diversity

Taxa Summary by Sample (Phylum)

Taxa summary by infant (genus)

Healthy term Infants (Hesla, et al. 2014)

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Bacteroidetes</th>
<th>Firmicutes</th>
<th>Proteobacteria</th>
<th>Actinobacteria</th>
<th>Other</th>
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</thead>
<tbody>
<tr>
<td>Hesla et al.</td>
<td>6</td>
<td>38</td>
<td>18</td>
<td>38</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Groer</td>
<td>30</td>
<td>7</td>
<td>60</td>
<td>9</td>
<td>.3</td>
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Looking for variables that explain alpha-diversity (linear models)

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>Pr(&gt;F)</th>
<th>R²</th>
<th>Adjusted R²</th>
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</thead>
<tbody>
<tr>
<td>Maternal BMI</td>
<td>3.48</td>
<td>0.06</td>
<td>0.009</td>
<td>0.006</td>
</tr>
<tr>
<td>Antibiotic X MOM</td>
<td>23.8</td>
<td>0.0002</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>Week 1</td>
<td>3.02</td>
<td>0.01</td>
<td>0.024</td>
<td>0.007</td>
</tr>
<tr>
<td>Week 2</td>
<td>14.4</td>
<td>0.0003</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Week 3</td>
<td>3.87</td>
<td>0.05</td>
<td>0.009</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Beta diversity

Effects of feeding
11 OTUS significantly enriched in formula fed infants

- Eg: Anaerococcus (p = 1.0 × 10^{-12})
Effects of preterm infant dysbiosis

- Gut inflammation
- Sepsis
- NEC
- Catch-up growth (SGA as well as preterm); obesity and the microbiome (dysbiosis: Firmicutes/Bacteroides)
- Long term effects in VLBWs: developmental, GI, autoimmune

Discussion and Future Work

1. Explore days on antibiotics and types of antibiotics in relationship to gut microbiome
2. Detailed exploration of time course
3. Metagenomic studies
4. Follow up studies on specific aims 2 and 3

Try Glimmix, Weight for age z-scores (Fenton)

- Not adjusted for any variable (gestational age is significant, sex is not)
- Quintic (5 parameters) is best fit with time

Weight for Height

- While the infants are losing weight for height across their NICIU stay, at 2-3 years of age their weight for height increases (ie; they are getting fatter)
Adjusted Weight for Height Z scores for 2 year olds

Discussion and Future Work

Acknowledgements

The “Frozen” team