THE DEVELOPING MICROBIOME: RELATIONSHIP TO HEALTH AND DISEASE

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Agenda

• Intestinal Microbe Host Interactions
• Preterm Birth.
• Neonatal Diseases, i.e., NEC.
• How we mess things up.
• C-section vs. vaginal Delivery
• Human Milk Microbiome
• The Brain
The Gut: Regional Differences

Neish, A. Gastroenterology vol. 136, No. 1, 2009
Mechanisms: Metabolomic (Bioreactor) Role

Gut bacteria and obesity
“Holy shit!”
Nov 12th 2009
From The Economist print edition
A new way of finding out how diet affects gut microbes

Turnbaugh, PJ Cell Host Microbe Volume 3, Issue 4, 17 April 2008, Pages 213-223
Toll-like receptors: ligands and signaling pathways

- dsRNA
- LPS
- Lipoprotein
- Flagellin
- Imidazoquinolines (anti-viral compounds)
- CpG DNA

TLR3, TLR4, TLR1 or TLR6, TLR2, TLR5, TLR7, TLR9

- TRAM
- TIRAP
- MyD88

- Adaptor
- IRF3
- NF-κB
- Transcription factor

Type I interferons (IFN-α/β) (antiviral activity)

Inflammatory cytokines
<table>
<thead>
<tr>
<th>Question</th>
<th>True</th>
<th>False</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amniotic fluid is sterile?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The placenta is sterile?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meconium is sterile?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Milk is Sterile?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Is there a Fetal Microbiome? Might this Relate to Prematurity

![LIFE Magazine cover](image)

![Graph showing correlation](image)

\[ r^2 = 0.42 \]
\[ P = 0.002 \]

The Placenta Harbors a Unique Microbiome

Kjersti Aagaard,¹,²,³* Jun Ma,¹,² Kathleen M. Antony,¹ Radhika Ganu,¹ Joseph Petrosino,⁴ James Versalovic⁵

Humans and their microbiomes have coevolved as a physiologic community composed of distinct body site niches with metabolic and antigenic diversity. The placental microbiome has not been robustly interrogated, despite recent demonstrations of intracellular bacteria with diverse metabolic and immune regulatory functions. A population-based cohort of placental specimens collected under sterile conditions from 320 subjects with extensive clinical data was established for comparative 16S ribosomal DNA–based and whole-genome shotgun (WGS) metagenomic studies. Identified taxa and their gene carriage patterns were compared to other human body site niches, including the oral, skin, airway (nasal), vaginal, and gut microbiomes from nonpregnant controls. We characterized a unique placental microbiome niche, composed of nonpathogenic commensal microbiota from the Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria phyla. In aggregate, the placental microbiome profiles were most akin (Bray-Curtis dissimilarity <0.3) to the human oral microbiome. 16S-based operational taxonomic unit analyses revealed associations of the placental microbiome with a remote history of antenatal infection (permutational multivariate analysis of variance, \( P = 0.006 \)), such as urinary tract infection in the first trimester, as well as with preterm birth <37 weeks \( (P = 0.001) \).
Meconium Analysis from Premature Infants

Gestational Age Differences

Ardissone, et al. PLOS One 2014
These sites allow for manipulation of the Fetal/Placental Microbiome
Gastric Aspirates: Term versus Preterm

Hypothesis: Could Swallowed “Infected” Amniotic Fluid Lead to Morbidity via an Intestine Derived Inflammatory Response?

LOCAL OR DISTAL ORGAN INJURY
- NEC
- Chronic lung disease
- Neuro-developmental delays.
- Premature Labor??
“Classic” NEC

Fecal microbiota: NEC

Mai V, Young C. PLOS One, May 2011

Controls, one week before diagnosis
- **Root; bacteria; actinobacteria**: 3.84%
- **Root; bacteria; bacteroidetes**: 8.06%
- **Root; bacteria; firmicutes**: 31.49%
- **Root; bacteria; others**: 0.01%
- **Root; bacteria; proteobacteria**: 56.40%
- **Root; bacteria; tennericutes**: 0.20%

Controls, <72h of diagnosis
- **Root; bacteria; actinobacteria**: 3.84%
- **Root; bacteria; bacteroidetes**: 8.06%
- **Root; bacteria; firmicutes**: 31.49%
- **Root; bacteria; proteobacteria**: 56.40%

Cases, one week before diagnosis
- **Root; bacteria; actinobacteria**: 0.47%
- **Root; bacteria; bacteroidetes**: 0.51%
- **Root; bacteria; firmicutes**: 60.58%
- **Root; bacteria; others**: 1.67%
- **Root; bacteria; proteobacteria**: 36.18%
- **Root; bacteria; tennericutes**: 0.48%

Cases, <72h of diagnosis
- **Root; bacteria; actinobacteria**: 0.18%
- **Root; bacteria; bacteroidetes**: 0.09%
- **Root; bacteria; firmicutes**: 28.79%
- **Root; bacteria; others**: 0.00%
- **Root; bacteria; proteobacteria**: 70.90%
- **Root; bacteria; tennericutes**: 0.03%
Odds Ratio of NEC with Increased Days on Antibiotics
Alexander, V.N. J. Pediatrics, Sept. 2011

Average length of Treatment increases odds by 50%
Epidemiology Studies of C-section vs. Vaginal Delivery

Higher odds of:

- Allergic Rhinitis
- Asthma
- Celiac Disease
- Type 1 Diabetes
- Gastroenteritis
- Allergies
- Obesity

*Neu J, Rushing, J. Clinics in Perinatology 2011
Vaginally delivered infants acquired bacterial communities resembling their own mother's vaginal microbiota and C-section infants harbored bacterial communities similar to those found on the skin surface. (Domínguez-Bello, MG. Proc Natl Acad Sci U S A. 2010 Jun 29;107(26):11971-5)
Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer

Maria G Dominguez-Bello, Kassandra M De Jesus-Laboy, Nan Shen, Laura M Cox, Amnon Amir, Antonio Gonzalez, Nicholas A Bokulich, Se Jin Song, Marina Hoashi, Juana I Rivera-Vinas, Keimari Mendez, Rob Knight & Jose C Clemente

Affiliations  |  Contributions  |  Corresponding authors

Nature Medicine (2016)  |  doi:10.1038/nm.4039
Received 03 July 2015  |  Accepted 22 December 2015  |  Published online 01 February 2016
Groups

18 dyads

Vaginal (7)

C/S (11)

Swabbed (4)

Not Swabbed (7)
Restitution of Vaginal Microbes

In an ACOG Practice Advisory, ACOG recommends against the practice of vaginal seeding. Vaginal seeding refers to using gauze or a swab to “transfer” vaginal fluids to an infant born via cesarean delivery to ameliorate the increased risk of autoimmune diseases, asthma, and allergic diseases found in children not exposed to vaginal microbiota. The presumption is that the reduced risk of autoimmune diseases, asthma, and allergic diseases in infants who are born vaginally is associated with their exposure to maternal vaginal microbiota. The Practice Advisory notes that there is no data about the safety and harms of vaginal seeding, but there is potential for harm from transfer of pathogens, such as group B streptococcus, herpes simplex virus, *Chlamydia trachomatis*, and *Neisseria gonorrhoea* from mother to child.
## Donor Human Milk after Holder Pasteurization

<table>
<thead>
<tr>
<th>Component</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Lipid</td>
<td>NA or ↓ 3.5-5.5%</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>↑ 83%</td>
</tr>
<tr>
<td>Lactose and oligosaccharides</td>
<td>NA</td>
</tr>
<tr>
<td>Total protein</td>
<td>NA or 4% reduction</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP)</td>
<td>↓ 99%</td>
</tr>
<tr>
<td>Bile salt-stimulated lipase (BSSL)</td>
<td>Abolished</td>
</tr>
<tr>
<td>Lactoferrin (LF)</td>
<td>NA or ↓ 44-91%</td>
</tr>
<tr>
<td>LF-iron-binding capacity</td>
<td>↓ 71%</td>
</tr>
<tr>
<td>IgA</td>
<td>NA or ↓ 20-60%</td>
</tr>
<tr>
<td>IgM</td>
<td>Abolished</td>
</tr>
<tr>
<td>IgG</td>
<td>↓ 34%</td>
</tr>
<tr>
<td>Lactoperoxidase (LP)</td>
<td>↓ 82%</td>
</tr>
<tr>
<td>LP activity</td>
<td>↓ 88%</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>↓ 24-60%</td>
</tr>
<tr>
<td>Lysozyme activity</td>
<td>NA or ↓ 65-85%</td>
</tr>
<tr>
<td>TGF-β</td>
<td>NA</td>
</tr>
<tr>
<td>IGF-I</td>
<td>↓ 39%</td>
</tr>
<tr>
<td>IGF-II</td>
<td>↓ 10%</td>
</tr>
<tr>
<td>IGFBPs</td>
<td>↓ 7-19%</td>
</tr>
<tr>
<td>EGF</td>
<td>NA</td>
</tr>
</tbody>
</table>
Breast milk microbes
Over time

Hunt, et al. PlosOne
2011

Figure 1. The community composition of the 15 most abundant bacterial genera in each of 3 milk samples from 16 subjects was diverse. The communities observed were found to be reasonably complex, and while consistent in composition over time for some subjects, a great deal of variation was observed over time in the samples of others.
doi:10.1371/journal.pone.0021313.g001
Bacterial Load over Lactational Stages: FusA Gene PCR

FIGURE 1 | Bacterial load over lactational stages. (A) Data show the median with ranges (maximum and minimum values for each group) of bacterial load at the three time points. C, colostrum samples (n = 19); T, transition milk samples (n = 20); M, mature milk samples (n = 17). (B) Lines show individual bacterial load for each mother at the three time points (n = 17).

Boix-Amaros, A. Frontiers in Microbiology. 20 April, 2016
Microbial Dose from Human Milk

- Assume intake of 800 ml/day
- Assume $10^{5-6}$ bacterial cells/ml
- This will provide $10^{7-8}$ bacterial cells (personalized?) daily, close to the dose in most probiotic studies.
Re-Faunation Experiments

- Donor Milk Refaunation: Adding mother’s own milk to pasteurized donor breast milk (DBM) to add back potentially beneficial microbes

Effect of separation on intestinal microbes

- Separation (MS) applied between days 4 and 19.
- Corticosterone levels measured day 20.
- Sacrificed at 70 days.
- Ussing Chamber permeability studies on intestine.

Maternal Separation: Rat Pups and Subsequent Intestinal Permeability

Barreau, F. Gut, 2004

Figure 1  Effect of neonatal stress on total gut (A) and colonic (B) paracellular permeability in 12 week old rats. Values are mean (SEM) (n=8 in each group). *p<0.05 between deprived and control rats.
Autism, Microbes and a Leaky Gut

- Mouse models of autism have GI barrier defects.
- This permeability defect can be corrected with the human commensal *Bacteroides fragilis*.
- This also ameliorates defects in communicative, stereotypic, anxiety-like and sensorimotor behaviors.
- These findings support a gut-microbiome-brain connection in ASD and identify a potential microbial therapy.

Take Home Messages

- Studies of the Microbiome are opening new avenues in our understanding of Health and Disease.
- The Fetal Maternal Unit is not sterile and prenatal exposure to microbes may be very important in subsequent development.
- Early life postnatal exposures such as mode of delivery, antibiotic use and diet are particularly important in that they can result in life-long changes in health and disease.
- Future studies will utilize multi-omic approaches to better delineate how microbes interact with the environment and the host in the cause and/or prevention of disease.