The Establishment of the Early Microbiome in Preterm Infants: Challenges & New Opportunities

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Rapid Succession of Early Exposures Influence Microbiome Development
Microbial Colonization Essential for Development of Innate Mucosal Immunity

- Environmental exposures
  - Diet (breast milk)
  - Bacterial colonization

MacPherson & Harris, Nature Reviews, 2004

- Poorly formed germinal centers
- Lamnia propria low CD4
- Reduced IgA in Peyer’s patches
Dysbiosis & Neonatal Mortality

- Commensal Bacteria
- Pathogenic Bacteria

① Altered Immune Ontogeny
② Dysregulated Inflammation
③ Impaired Organogenesis

- Necrotizing Enterocolitis
- Chronic Lung Disease
- Sepsis
Characterization of the Microbiome Promotes Practice Changes

- Maternal Medications
- Route of delivery
- Restore the microbiome
Dr. Maria Gloria Dominguez-Bello, associate professor in the Human Microbiome Program at the NYU School of Medicine
http://commonhealth.wbur.org/2014/06/birth-canal-bacteria-c-section
Characterization of the Microbiome Promotes Practice Changes

- Maternal Medications
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- Restore the microbiome

- Skin-to-Skin

- Breast Feeding
- Immuno-nutrients
Human Milk Oligosaccharides

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- Skin-to-Skin
- Breast Feeding
- Immuno-nutrients
- Handling/Cohort Practices
- Minimize exposure – Abx, H2 blockers
Common Medications Influence the Microbiome & Increase Risk of Disease


Figure 2. Simpson diversity index depicted in relation to antibiotic receipt (No, 0 days; brief, 1-4 days; or intensive antibiotics, 5-7 days) during the first 3 weeks of life.
Challenges for Personalized Medicine: Population Overlap vs. Unique Individualism
Population Differences but Significant Overlap


Figure 2. Simpson diversity index depicted in relation to antibiotic receipt (No, 0 days; brief, 1-4 days; or intensive antibiotics, 5-7 days) during the first 3 weeks of life.
Population Differences but Unique Individual Signatures

Population Differences but Unique Individual Signatures

Martin et al PAS 2012; and work in progress
Integration of the Microbiome & Omics

Postnatal Intestinal Host Responses & Risk of Neonatal Disease

NICU Interventions & Nutritional Practices
- Medications
- Lack of Enteral Substrate (NPO)
- Breast Milk
- Formula
- Bovine Milk-based Fortifiers
- Human Milk-based Fortifiers

Maladaptive Intestinal Development
- Altered Immunity
- Dysregulated Inflammation

Disrupted Barrier Defense
- Local Intestinal Injury

Systemic Consequences
- Distal Organ Damage
Influence of Diet on Intestinal Gene Expression

- Microbiome
- Epithelial Transcriptome
Non-invasive analysis of intestinal development in preterm and term infants using RNA-Sequencing

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Hypothesis

• Optimal development of the regulatory functions of immunity and inflammation in the intestinal epithelium is vital for infant health
  – Our understanding in this area has been limited by an inability to directly assess epithelial cell biology in the newborn intestine.

• We hypothesize that non-invasive transcriptomics from isolated epithelial cells in fecal samples will allow for reliable, robust measures to be applied to precisely identify the intestinal developmental pathways that are modulated by diet.

Methods

1. Fecal samples placed into RNALater to preserve RNA
   - PolyA1 RNA isolated from host epithelial cells
   - PolyA1 RNA converted to cDNA
   - Illumina libraries created

2. Microbial Communities
   - Sloughed Host Epithelium
     - Lyse
     - Eukaryotic Poly-A mRNA Selection
     - Spike-in ERCC Controls Added
     - Conversion to cDNA
     - SPIA Amplification, Fragmentation, Size Selection, Library Preparation

3. Perform Network and Pathway Analysis
   - Perform Differential Expression Tests
   - Quantify ERCC and Human read counts per gene/spike-in
   - Map against multiple references
     - Human
     - Microbial
     - Viral
     - Fungal
     - Protozoan
     - Ribosomal
     - Mitochondrial
   - Raw Reads
     - SW1-2921
     - acacctgat
     - SW1-2922
tgtgcgca...

4. Sequencing on Illumina GAIIx and HiSeq 2000 platforms

5. Pathway analysis (Ingenuity Pathway Analysis)

6. Gene expression with RT PCR confirmation

Summary of Differentially Expressed Genes

• 171 genes were differentially expressed between PT and FT infants

• Main pathway maps identified were:
  • Lipid Metabolism
  • Cellular Movement
  • Hematological System Development and Function
  • Immune Cell Trafficking
  • Small Molecule Biochemistry
  • Molecular Transport

Pathway Analysis – Preterm vs. Term
Lipid Metabolism & Immunity/Inflammation

Pathway Analysis – Clinical Significance

Lipid Metabolism
- Impaired lipid hydrolysis & absorption
- Increased acylcarnitine levels
- Decreased carnitine levels

Immune Function
- Dysregulated inflammatory response
- Increased pro-inflammatory protein expression
- Decreased expression of negative regulators

Cellular Function
- Balance of cell proliferation (organogenesis), homeostasis, apoptosis & repair

Implications

• Whole-genome sequencing of stool-derived RNA allows for sequential, longitudinal monitoring of gut gene expression in real-time in response to dietary or therapeutic interventions.

• Up-regulation of genes of lipid metabolism, immune cell function and inflammation pathways in preterm infants support the hypothesis that altered lipid metabolism and its role in immunity and inflammation may account for the increased risk of NEC in preterm infants.

• The potential of this technology can be enhanced by including additional systems biology data, including metabolomics and microbiome/metagenomics, to further define intestinal host responses to their environment.

Conclusions

• The newborn infant undergoes rapid exogenous exposures that influence microbial colonization

• The preterm infant is particularly vulnerable to dysbiosis and subsequent morbidities

• Unique, individual microbiome signatures represents challenges for translation to population health

• Integration with –omic strategies may allow for translation to personalized medicine; while uncovering predictable patterns for applied population strategies
Acknowledgements

- Program for Faculty Development and Diversity of Harvard Catalyst, Harvard Clinical and Translational Science Center, National Center for Research Resources (award UL1 RR 025758)
- Texas A&M AgriLife Research
- NIH grants CA129444, U01CA162077, HD61929, R25TCA090301, P30ES023512,
- Hatch project ILLU-971-346 through the Division of Nutritional Sciences Vision 20/20 program
- USDA–NIFA Grant Designing Foods for Health 2010-34402-20875
Thank You!

I appreciate your attention.
Other References

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