Microbes and Neonatal Immunity
Hitesh Deshmukh MD, PhD
September 18th 2015

Overview

Microbes within us

How is the neonatal microbiome assembled

How our modern obstetric and medical practices are altering the neonatal microbiome

Consequences of disrupting the microbiome

Microbiome directs the development of neonate’s immune system

Microbes within Us
How many microbes?

Contains 10 times more microbial cells than human cells.

How many microbes do we carry?

Contains 10 times more microbial cells than human cells.

How many microbes do we carry?
How many microbes do we carry?

**Microbiota:** All microbial taxa associated with humans

**Microbiome:** Catalog of all these microbes and their genomes

_Evolution of Mammals and Their Gut Microbes_

_Horizontal gene transfer in eukaryotic evolution_

_Not Accidental_
Why care about these microbes?

Why are microbes important to us?

Why are microbes important to us?
What are these microbes?

Human Microbiome Project

Microbial Map of our body

The Neonatal Microbiome

- How is the neonatal microbiome assembled?
- What is the source of founding microbes?
- What factors influence the assembly?
- What happens when assembly is disrupted?

Mode of Birth

Sterile womb?

Mode of Delivery

Dominguez-Bello et al. 2011 PNAS (107):11971

Jakobson et al. 2014 Gut (63):559

Breast Milk

Development and Differences of Intestinal Flora in the Neonatal Period in Breast-Fed and Bottle-Fed Infants
Hajime Tsuchida, MD, Kenichi Iwaki, MD, and Kazu Fujie, MD
PEDIATRICS Vol. 73 No. 3 September 1984 DOI

Breast Milk: Is there a BM microbiome?

Distinct from other niches
Changes with time
Heavier the mother: Less the bacteria
Less diverse in mothers undergoing caesarean section

Gestational Age

24 weeks 28 weeks 32 weeks

Bacilli > Gammaproteobacteria > Clostridia

La Rosa et al. 2014 PNAS (111):12522

Assembly of Neonatal Microbiome

Koenig et al. 2011 PNAS (108):4578

ABX in perinatal period

C-sections are increasing

> 50 % colonized with GBS
ABX use is common during birthing process

C-sections are increasing

> 50% colonized with GBS

Adapted from Persaud et al. 2015 J Mat-Fetal & Neonat 28:10-1190

<table>
<thead>
<tr>
<th>Indication</th>
<th>Prevalence (n=440)</th>
<th>Mother colonized</th>
<th>ABX use</th>
<th>Mother colonized</th>
<th>Neonate colonized</th>
</tr>
</thead>
<tbody>
<tr>
<td>VBAC</td>
<td>76.5%</td>
<td>26.2%</td>
<td>14.9%</td>
<td>26.2%</td>
<td>3.9%</td>
</tr>
<tr>
<td>Elective CS</td>
<td>19.2%</td>
<td>32.6%</td>
<td>23.1%</td>
<td>32.6%</td>
<td>7.3%</td>
</tr>
<tr>
<td>Group B streptococcus</td>
<td>25 (57)</td>
<td>7.9%</td>
<td>40.0%</td>
<td>40.0%</td>
<td>10.0%</td>
</tr>
<tr>
<td>Preterm (n=120)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perinatal (n=120)</td>
<td></td>
<td></td>
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</table>

Preterm neonatal mortality:

<table>
<thead>
<tr>
<th>N (include CS, GBS)</th>
<th>176 (223)</th>
<th>20.7%</th>
<th>14</th>
<th>6.3%</th>
<th>9</th>
<th>4.1%</th>
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Perinatal ABX use and Microbiome

High-Throughput Sequencing Reveals the Incomplete, Short-Term Recovery of Infant Gut Microbiota following Perinatal Antibiotic Treatment with Ampicillin and Gentamicin

Influence of intrapartum antibiotic prophylaxis against group B Streptococcus on the early newborn gut composition and evaluation of the anti-streplococcal activity of rifampicin and amoxicillin.

Effects of Intrapartum Penicillin Prophylaxis on Intestinal Bacterial Colonization in Infants

Perinatal ABX during birthing process

Adapted from Journal of Pediatrics 166 (3) 538 2015

Perinatal ABX use alters the neonate's microbiome

Adapted from Persaud et al. 2015 J Mat-Fetal & Neonat 28:10-1190
ABX use in neonates

<table>
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<tr>
<th>Medication</th>
<th>Frequency</th>
<th>Duration (hr)</th>
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<tr>
<td>Vancomycin</td>
<td>171/360</td>
<td>475</td>
</tr>
<tr>
<td>Meropenem</td>
<td>581/102</td>
<td>239</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>328/428</td>
<td>123</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>67/276</td>
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<td>47/276</td>
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Adapted from Persaud et al. 2015 J Mat-Fetal & Neonat 28:10

Recovery after ABX use is delayed

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Adapted from: Schulman et al Pediatrics 1135 (5) 2015

**TABLE 1**

| NOD Level of Care | Total Number of NODs | Number of NODs AUR Quantile 1 | Number of NODs AUR Quantile 2 | Number of NODs AUR Quantile 3 | Number of NODs AUR Quantile 4 | Lowest AUR Value | Highest AUR Value | Range of AUR | AUR Quartile 1 | AUR Quartile 2 | AUR Quartile 3 | AUR Quartile 4 | Range of AUR (Multihour to Highest Value) |
|--------------------|----------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|------------------|------------------|--------------|----------------|----------------|----------------|----------------|----------------|-------------------------------------------|
| All NODA           | 127                  | 32                             | 2                              | 5                              | 1                             | 2.4              | 97.1            | 47           | 46            | 36            | 21            | 17             | 31             | 40                         |
| Region             | 21                   | 4                              | 7                              | 6                              | 4                             | 3.6              | 63              | 17           | 14            | 10            | 9              | 6              | 10             | 7                         |
| Community          | 79                   | 25                             | 14                             | 17                             | 10                            | 3.9              | 76              | 8             | 42            | 11            | 8              | 3              | 12             | 7                         |
| Intermediate       | 14                   | 5                              | 7                              | 6                              | 3                             | 3.1              | 69              | 3             | 15            | 6             | 3              | 2              | 12             | 2                         |
| Non-COB            | 13                   | 2                              | 3                              | 2                              | 1                             | 3.4              | 73.7            | 3             | 17            | 6             | 3              | 2              | 12             | 5                         |

Adapted from: Schulman et al Pediatrics 1135 (5) 2015
Recovery after ABX use

Even short courses of ABX disrupt microbiome
Mouse ≠ Human
Microbiome assembly in Neonatal Mice

PC1 (21%)  
PC2 (26%)

Intestine  
Vagina  
Skin  
Neonate

Microbiome assembly in Neonatal Mice

PC1 (21%)  
PC2 (26%)

Intestine  
Vagina  
Skin  
Neonate

Day 0

Frequency of 16S DNA

0  50  100

Microbiome assembly in Neonatal Mice

PC1 (21%)  
PC2 (26%)

Intestine  
Vagina  
Skin  
Neonate

Day 0  Day 3

Frequency of 16S DNA

0  50  100

Samples: 
- Control
- P0
- P3
- Uncultured
- Thrombomycetes

Microbiome assembly in Neonatal Mice

PC1 (21%)  
PC2 (26%)

Intestine  
Vagina  
Skin  
Neonate

Day 0

Frequency of 16S DNA

0  50  100
Microbiome assembly in Neonatal Mice

Bacilli > Gammaproteobacteria > Clostridia

Antibiotics disrupt the assembly of murine neonatal microbiome
Ontogeny of Immune System

• Immature adaptive immune response
• Th2 Bias
• Primacy of innate immune system

Ontogeny of innate immune cells

• How do innate immune cells develop in neonates
• Neutrophils: Primary effector cells of granulocyte lineage in neonates
• Innate lymphoid cells: Primary effector cells of lymphoid lineage in neonates
• Use microbes as tool to study the development of neutrophils and innate lymphoid cells.
Adapted from: Deshmukh et al Nature Med 2014 (20) 5
Restoration of commensal bacteria restores host resistance.
Lineage Negative Lymphocytes

RORγT
Signature transcription factor of ILC3
WT neonate (P3)

Rem: iDT

neonate (P3) + DT

Percent survival

Time (h) post infection

iDT (DT) (n=6)
iDT (DT) + Adoptive transfer (n=6)

WT + Adoptive transfer (n=5)

6 h post infection

Control

No ABX

ABX

6 h post infection

Control

No ABX

ABX

Number of cells

AT T cells

ILC3

No ABX

ABX

Control

6 h post infection

No ABX

ABX

Control

6 h post infection
Defect in migration of ILC3
Adapted from Annual Reviews in Immunology 32:659 2014

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Adapted from Annual Reviews in Immunology 32:659 2014
Dendritic cells imprint lung CCR4 expression and lung homing on ILC3
Hacking the microbiome

Prebiotics: Fertilizer for lawn of gut microbes
Glycans and Fructans → SCFA and Butyrate
Breast milk is the best prebiotic
Probiotics: Reseed the lawn selectively
Fecal transplants: Time to rip the lawn and lay fresh sod
Vaccines
What is coming?


What is coming?

ClinicalTrials.gov
A service of the U.S. National Institutes of Health

Preterm Infant Gut (PINGU) - a Norwegian Multi Centre Study

This study is currently recruiting participants. See Contacts and Locations.
Verified June 2015 by University Hospital of North Norway

ClinicalTrials.gov Identifier: NCT02197486