Development of the Early Gut Microbiome in Health and Disease

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Disclosures

• Financial COI : none
• Off-label uses of medications or devices: none
• Funding sources:
  NIH
  FNIH
  Children’s Discovery Institute
  (St. Louis Children’s Hospital and Washington University)
Meconium fills gut at birth

Meconium passes, culturable bacteria expand
Early in life microbes affect host later in life

Colonization of GF mice during a specific interval permanently affects systemic immune responses


Age sensitive colonization with commensal microbes establishes iNKT cell tolerance in GF mice (Olszak, et al, Science 336:489)

Earliest in Life Colonization in Humans: Ideal Conditions

• High frequency sampling to define rapidly changing microbial populations
• Many subjects: data should be generalizable
• ‘Managed’ environment: reduces exogenous drivers
• Accurate host metadata to control for different variables to the extent possible
The Premature Infant: An Instructive Host in an Opportune Environment

Managed ecosphere (no pets, few handlers, few oral antibiotics)

Hospitalized in a single unit for 2-4 months

Extensive metadata

Subjects & Specimens

• 58 preterm infants, GA 23-33 weeks
• All stools up to DOL 30 sequenced (V3-V5, 454), then every 3 days thereafter
• 922 samples
• 7190 (IQR = 5244-9396) reads/subject
Bacilli predominate at birth, then diminish
Clostridia slowly rise
Gammaproteobacteria abundant and persistent
Bacilli, Clostridia, Gammaproteobacteria: 93% of all taxa on all days

3 Classes Predominate

<table>
<thead>
<tr>
<th>Class</th>
<th>Cohort</th>
<th>Species represented</th>
<th>Older children and adults*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacilli</td>
<td>19%</td>
<td>34</td>
<td>1-5%</td>
</tr>
<tr>
<td>Gammaproteobacteria</td>
<td>54%</td>
<td>52</td>
<td>0.1-1%</td>
</tr>
<tr>
<td>Clostridia/Negativicutes</td>
<td>19%</td>
<td>40</td>
<td>45-80%</td>
</tr>
</tbody>
</table>

Subject A – Class Level

Bacilli
Enterococci, Staphylococci
Gammaproteobacteria
*Enterobacteriaceae* (Escherichia, Klebsiella, Enterobacter)

Clostridia/Negativicutes
*Clostridium sensu stricto, Clostridium XI* Veillonella
Single sample variations

Clostridia gain abundance slower in more premature infants

PNAS 2014;111(34):12522-7
Progression from Bacilli → Gammaproteobacteria → Clostridia is not smooth

Abrupt changes punctuate the progression of community assembly
10/19/15

- DOL predicts community structure
- GA at birth predicts rate of Clostridial accrual
- Antibiotics: slight ↑Gammaproteobacteria, ↓Clostridia
- Mode of delivery, feeds do not drive this progression

Patterned progression in St. Louis …
... replicated in Oklahoma and Kentucky

Succession Ecology


Premature infant gut harbors multidrug resistant organisms

Molly Gibson, Gautam Dantas (in review)
Functional resistance-conferring genes are in NCBI database, but not in known ARG databases

Eukaryotic virome expands, phage virome contracts

Molly Gibson, Gautam Dantas (in review)

Efrem Lin, David Wang, Lori Holtz
Phage and Bacteria are Inversely Related: Predator:Prey Model

Necrotizing Enterocolitis (NEC)
Acute inflammation and necrosis, sudden onset

Efrem Lin, David Wang, Lori Holtz

4-10% of VLBW (<1500 g BW), 30-40% fatal
Major cause of intestinal failure, and neurocognitive impairment
Treatment remains removal of affected bowel
NEC: One name, many contributors

- Genetics
- Microbiome
- Innate immune system
- Substrate
- Inflammation
- Transfusions
- Ischemia

Summary of Enrollment

- 957 infants at risk (489 SLCH, 276 OK, 207 KY)
- 46 cases of NEC (Stage II or III) after removing subjects with inadequate specimens, or heart lesions
- Controls selected based on gestational age at birth (+/- 1 week), birth weight (+/- 100 g), and closest in time
St. Louis Cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>28</td>
<td>94</td>
</tr>
<tr>
<td>BW (g)</td>
<td>795</td>
<td>940</td>
</tr>
<tr>
<td>GA</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>No. of stools analyzed pre-NEC (median)</td>
<td>12.5</td>
<td>21.5</td>
</tr>
<tr>
<td>Died before discharge</td>
<td>9 (32%)</td>
<td>1 (1.1%)</td>
</tr>
</tbody>
</table>

Other variables: race, sex, ethnicity, APGAR scores, CRIB II, vaginal delivery, abx, human milk, inotropes, blood transfusion

28 cases, 94 controls, 2293 stools, 15,112,935 reads
7.4 gigabases of bacterial sequences
Pre-NEC microbes differ from controls
but effect apparent only > 30 days of age
...and not for infants born at ≥ 27 weeks’ gestation

Mixed Model Analysis
(takes into account multiple measurements from individuals)
Time interval, GA at birth, ABX, milk, route of delivery: small or inconsistent associations

<table>
<thead>
<tr>
<th>TIME-BY-NEC INTERACTION</th>
<th>Bacilli</th>
<th>Gammaproteobacteria</th>
<th>Clostridia</th>
<th>Negativicutes</th>
<th>Negativicutes + Clostridia</th>
</tr>
</thead>
<tbody>
<tr>
<td>All GA</td>
<td></td>
<td>0.148</td>
<td>-0.021</td>
<td>-0.061</td>
<td>-0.001</td>
</tr>
<tr>
<td>&lt; 27 weeks</td>
<td>-0.062</td>
<td>0.195</td>
<td>-0.017</td>
<td>-0.042</td>
<td>-0.060</td>
</tr>
<tr>
<td>weeks</td>
<td></td>
<td></td>
<td>0.007</td>
<td>0.030</td>
<td>0.021</td>
</tr>
<tr>
<td>≥ 27 weeks</td>
<td>0.022</td>
<td>0.144</td>
<td>-0.059</td>
<td>-0.051</td>
<td>-0.159</td>
</tr>
<tr>
<td>weeks</td>
<td>0.034</td>
<td>0.228</td>
<td>0.271</td>
<td>0.094</td>
<td>0.028</td>
</tr>
</tbody>
</table>
Interim Conclusions

- Gut microbial community structure predisposes to NEC
- Positively associated with Gammaproteobacteria
- Negatively associated with anaerobes

Might Microbial Management Prevent NEC? (or at least some of it)
Microbiologically Informed: Cohort and Timing of Treatment

The Team: Barb Warner, Participating Families, NICU Staff

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Molly Gibson

Wang and Holtz Labs
Efrem Lin

NIAID/NICH, UH3 AI083265-02, FNIH