The Developing Intestinal Microbial Ecosystem: Relationship to Subsequent Health and Disease

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National Human Genome Research Institute, NIH

The Microbiome

- **Microbiome** - The Microbiome is the full collection of microbes (bacteria, fungi, viruses, etc.) that naturally exist within the human body.
- **Goal of NIH Microbiome Roadmap** - Understanding whether changes in the human microbiome can be correlated with changes in human health.

**Extra! Scientists Discover a New Organ in Humans!**


**Intestinal Microflora**

- **stomach**
  - bacterial numbers: c. 10^4/ml contents
  - *e.g.* *Helicobacter pylori*

- **small intestine**
  - bacterial numbers: c. 10^9/ml contents
  - *e.g.* lactobacilli, Gram-positive cocci

- **colon**
  - bacterial numbers: c. 10^{12}/g contents
  - *bacteria*: bifidobacteria, clostridia, peptostreptococci, fusobacteria, lactobacilli, enterobacteria, enterococci, eubacteria, methanogens, sulphate reducers etc.

A. Mullard, Nature 2008;453:578-580
**Bacterial 16S RNA**


**Examples of Non-Culture Based Techniques**

Mashvildaze, Neu and Mai
Nutrition Reviews, 2009

**Bacterial 16SrRNA Gene Sequences From Intestinal Microbiota of Animals**


**Site Specific Distribution of Bacterial Phyla in Healthy Humans**


**Agenda**

- Microecology of the Developing GI Tract.
- Relationship of Intestinal Microbes to Innate Immune system and Inflammatory pathways in the GI tract.
- Relationship of microbial ecology the fetus to intestinal inflammation and prematurity.
- Relationship of Intestinal Microbes to Protection of the GI tract.
- The Role of Probiotics (Live vs. Dead vs. Components), Prebiotics and “Postbiotics”.

Don’t mess with my Microbiome!
Commensal Microbes: Beneficial Effects for the Host:

- Nutrient metabolism
- Tissue development
- Resistance to colonization with pathogens
- Maintenance of intestinal “homeostasis”

Development of Intestinal Angiogenesis With Microbes

- Villi from (P14) versus (P28) conventionally raised mice.
- Capillary networks are stained green (FITC).

Stappenback, Hooper and Gordon, PNAS, 2002

HOW DO BACTERIA PREVENT DAMAGE TO THE INTESTINAL TRACT?

Stappenback, Hooper and Gordon, PNAS, 2002
**Bacteria and Immune Activation**


**Tales From the Crypt-Paneth Cell**

defensins and angiogenins

Ganz, T. Nature Immunology, 2003

**Toll-like receptors: ligands and signaling pathway**

**TLRs, Microbes and Protection**

- Oral dextran sulfate sodium (DSS) to induce colonic injury
- Deplete mice of intestinal microflora
- Reintroduce commensals and commensal products

Rakoff-Nahoum S et al. Cell 2004

**TLR Knockouts and Survival and Weight Loss**

Rakoff-Nahoum S et al. Cell 2004

**Could Antibiotics lead to unforeseen consequences?**
Commensal microflora are required for protection from mortality due to colonic epithelial injury

Depletion of all commensals: increased mortality

Rakoff-Nahoum S et al. Cell 2004

Recognition of commensal ligands required for protection from colon injury

Implications

- Gut epithelium and immune system do not simply tolerate commensal microflora but are dependent on them
- Manipulation of the intestinal microflora might lead to major consequences.

Microbial Ecology of the Uterus and Fetal GI tract: Nexus with Innate and Adaptive Immunity and Disease

Prematurity

- 4 million U.S. births per year
- 12% (480,000) premature
- 2% (80,000) < 1.5 kg BW (3 lbs) (VLBW or very low birthweight)
Relationship of Amniotic Infection (without ruptured membranes) to Prematurity

- Quantitative amniotic fluid cultures were performed on 12 patients delivered of premature infants.
- Only 2 of the 12 had premature rupture of membranes.
- Seven of the 10 women with in preterm labor who had intact membranes had colony counts greater than 1,000 per ml.

Bobbit, JR: J. Reproductive Medicine, 1977

Goldenberg, RL NEJM, 2000

Microbial Identification Techniques: Culture vs. PCR

Outcomes According to PCR and Culture Results

PCR and Culture Results with AF Markers of Infection
Infants born preterm have different concentrations of many inflammatory markers in blood drawn several days after birth compared to infants born at term. The degree of association between the levels of inflammatory markers and preterm birth correlated with the degree of prematurity. These results support the hypothesis that the fetus is an important source of inflammation and may play a role in the causes of preterm birth.

Don’t mess with my Microbiome!

TOP TEN LIST OF NICU MEDICATIONS

<table>
<thead>
<tr>
<th>Medication Name</th>
<th>Frequency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>18.7%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>17%</td>
</tr>
<tr>
<td>Nosoxin</td>
<td>12%</td>
</tr>
<tr>
<td>Vitamin Multivitamin</td>
<td>0.2%</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.8%</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.5%</td>
</tr>
<tr>
<td>Fosamprenopyridine</td>
<td>0.2%</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.1%</td>
</tr>
<tr>
<td>Imipenem/Sarritanamide</td>
<td>0.1%</td>
</tr>
</tbody>
</table>


**Antibiotics in Premature Infants**

- Ampicillin and aminoglycoside used in >90% of premature less than 30 weeks gestation for at least 2 days: **WHY?**
  - Can’t tell the difference between RDS and pneumonia.
  - Premature delivery might be caused by infection.
  - Want to prevent infections because of high susceptibility due to immunologic immaturity.
  - Hardware (ET and OG tubes, umbilical lines, etc.).
  - Sensitivity of blood cultures suboptimal

**Delayed Colonization and “Unnatural Selection”: Why Worry?**

- The first colonization of the intestine is one of the most profound immunological exposures faced by the newborn infant.
- Crosstalk induces gene expression in both the epithelium and the immune system.
- Niches are formed as part of a potentially long lasting biofilm located within the luminal glycocalyx.

**What Does Neonatal Antibiotic Treatment Do To Gastrointestinal Tract Development?**

- 100 mg/kg/d Clamoxyl (Amoxicillin) compared with saline control.
- All bacteria were significantly reduced especially Lactobacillus, mainly in colon.
- Affymetrix gene microarrays performed.
- 30-30% of the genes undergoing maturational changes showed modulation by the antibiotic so that their normal pattern of maturation was either accelerated or slowed down.
- 0HC genes markedly affected—required for tolerization to luminal antigens.


**Intravenous Antibiotics and NEC**

Table S9. Multivariable Logistic Regression: Antibiotic Duration and NEC or Death

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Duration of Initial Empirical Antibiotics (odds per day) OR (95% CI)</th>
<th>p-value</th>
<th>Prolonged Initial Empirical Antibiotics (≤ 5 days) OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC or Death</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N=3353</td>
<td>N without NEC=454</td>
<td>1.09 (1.02, 1.17)</td>
<td>&lt;0.01</td>
<td>1.30 (1.15, 1.54)</td>
</tr>
<tr>
<td>NEC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N=3095</td>
<td>N without NEC=349</td>
<td>1.07 (1.04, 1.11)</td>
<td>&lt;0.001</td>
<td>1.21 (0.99, 1.51)</td>
</tr>
<tr>
<td>Death</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N=3682</td>
<td>N without NEC=319</td>
<td>1.16 (1.08, 1.25)</td>
<td>&lt;0.001</td>
<td>1.46 (1.15, 1.85)</td>
</tr>
</tbody>
</table>

Cotten C.M. Pediatric Research 2007
Persistent Biofilm Formation: Should we Worry?

Probiotic Studies in Prematures: Randomized Trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention</th>
<th>Control</th>
<th>Relative Risk</th>
<th>Risk Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mccrea</td>
<td>1.7/2 (4)</td>
<td>1.7/2</td>
<td>0.12</td>
<td>0.31</td>
</tr>
<tr>
<td>Scholten</td>
<td>1.8/2 (2)</td>
<td>1.8/2</td>
<td>0.12</td>
<td>0.31</td>
</tr>
<tr>
<td>Hess</td>
<td>1.2/2 (1)</td>
<td>1.2/2</td>
<td>0.12</td>
<td>0.31</td>
</tr>
</tbody>
</table>


Alternatives to Live Probiotic Microbes

- Inactivated by heat or UV irradiation.
- Pre and Post-biotics
- Toll receptor agonists
  - LPS (TLR-4)
  - LTA (TLR-2)
  - Flagellin (TLR-5)
  - CpG nucleotide (TLR-9)

Potential Dark Side of Prebiotics

- Promoting growth of NICU bad bugs.
- Increased bacterial translocation
- Unknown long term effects of early manipulation of microbiome.

Prebiotics: Problems in Premature

- Prebiotics on their own can only enhance the growth of bacteria already present in the gut.
- If health promoting species are not present to begin with, the prebiotic is unlikely to be effective.
Increased Bacterial Translocation in Rats Fed Using Pup in Cup

Prevention with Mother’s Milk: NEC in Premature Infants (UK)

In-Hospital Diet All Cases Confirmed Cases
Formulas Only (n=236) 24 (10.2%) 17 (7.2%)  
Formulas plus  
Mother’s Milk (n=437) 16 (3.7%) 11 (2.5%)  
Human Milk (n=253) 11 (4.3%) 3 (1.2%)  

Lucas & Cole, Lancet 1980;336:1519-23

Breast Milk Microbial Origin: Maternal Intestine?

Relation Between Infection, Antibiotic Usage and Diseases

Perez, PF. Pediatrics 2007;119;e724-e732

GNP vs. MS, Type 1 diabetes and Asthma

Bach, JF: NEJM 2002

Bach, JF. NEJM 2002
Th1 and Th2 Diseases

However, the incidence of TH-1 predominant diseases (autoimmune, e.g. type 1 diabetes) and TH-2 (allergy, atopy, asthma) have both increased.

“Old Friends” Hypothesis

Are there long term effects of intestinal microbiome manipulation?

Probiotics and atopic dermatitis

Probiotics: Late Complications

- LGG administered to mothers prior to delivery and then to the infants shortly after delivery: decreased atopic dermatitis in the group receiving LGG, but more of allergic rhinitis and asthma in the Lactobacillus GG group at 7 years
- At 2 years of age, LGG did not result in differences in atopic dermatitis, but there was a statistically significant increase in wheezing bronchitis (26% vs. 9%) in the LGG-treated group
- At the age of 12 months, the rate of sensitization to common allergens was significantly higher in the probiotic group

CS15  Should this read the incidence of.....has increased rather than the increase in....has increased
Computer Support, 5/19/2005
Don’t mess with my Microbiome!

Take Home Messages

- There is a close relationship between intestinal microbes and development of the GI tract, immunity, and other systems.
- Many microbes cannot readily be detected by usual culture-based techniques.
- Microbes in amniotic fluid are related to premature delivery via an inflammatory response.
- We still have a lot to learn about intestinal microbes and their interactions with the developing host to manipulate them safely, especially in select groups, e.g. premature infants.