Identification of host-microbial interaction networks that mediate intestinal epithelial barrier maturation

David R. Hill, PhD

Basic & Translational GI Research Conference
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How is host-microbe mutualism in the gut established and maintained?

- *Tissue maturation*
- *Innate defense*
- *Barrier resilience*

Multicellular life did not take over the globe by combat, but by networking

- Dr. Lynn Margulis

David R. Hill, PhD
Window of opportunity for microbiota modulation from gestation to childhood.

**Prenatal factors:**
- Placenta

**Neonatal factors:**
- Mode of delivery
- Gestational age

**Postnatal factors:**
- Feeding: breast-milk vs. formula
- Geographical location
- Family members
- Host interactions
- Maternal diet
- Weaning

**Gestation**

**Birth**

**Infancy**

**Toddler & childhood**

Bacterial succession and host sculpting shape the developing intestine and its microbiota.

Rook et al 2017 *The Lancet*

Bevins and Salzma 2011 *CMLS*
Infant health status and microbiota establishment

Good bacteria
- Bifidobacterium species
- Escherichia coli
- Faecalibacterium prausnitzii
- Lactobacillus species

Bad bacteria
- Enterococcus faecalis
- Methanobrevibacter smithii
- Clostridium difficile
- Campylobacter

Health status
- Infection
- NEC
- Atopy
- Diabetes
- IBD/IBS

Disease status

Microbiota Establishment
How do differences in microbial colonization produce different health and developmental outcomes?

**Infant health status and microbiota establishment**

**Good bacteria**
- *Bifidobacterium* species
- *Escherichia coli*
- *Faecalibacterium prausnitzii*
- *Lactobacillus* species

**Bad bacteria**
- *Enterococcus faecalis*
- *Methanobrevibacter smithii*
- *Clostridium difficile*
- *Campylobacter*

Infection
- NEC
- Atopy
- Diabetes

Bacterial interactions with epithelium are central to understanding the mechanistic causes of microbiota-associated diseases.
Comparing intestinal model systems

Young VB. Old and new models for studying host-microbe interactions in health and disease: C. difficile as an example. Am J Physiol Gastrointest Liver Physiol. 2017
Directed differentiation of pluripotent stem cells into intestinal tissue

Directed differentiation of pluripotent stem cells into intestinal tissue


*In vitro* organoid culture
Stem-cell derived organoids are capable of maturation into adult-like structures upon transplantation

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Methodology for colonizing HIOs with live bacteria

**DAY -30 to -1**: Prepare HIOs according to McCraken et al (2011) and plate 1 HIO per well (24-well plate) in 50 µl Matrigel and 500 µl ENR media

**DAY -1**: Culture E. coli from glycerol stock in LB broth overnight at 37°C

**DAY 0**: Microinject approximately 1 µL of the overnight E. coli culture (diluted in PBS) into the HIO lumen

**After microinjection**: Remove ENR media and wash 1X with sterile PBS.

**0-1 h post-microinjection**: Add ENR media containing Pen/Strep mix and incubate at 37°C for 1 hour

**1 h post-microinjection**: Remove ENR containing antibiotics and wash 1X with sterile PBS.

Replace fresh ENR without antibiotics and culture at 37°C and 5% CO₂

Hill et al. (2017). eLife
Hill et al. (2017). JoVE
Stable bacterial colonization promotes expression of gene sets critical for epithelial barrier function

Hill et al. eLife 2017
Measuring epithelial barrier permeability in HIOs

**E. coli** colonization enhances epithelial barrier resilience

- TNFα and IFNy released during intestinal inflammation increases epithelial barrier permeability

- Control HIOs or HIO pre-colonized with *E. coli* treated with TNFα and IFNy for 24 hrs

- Epithelial barrier integrity is maintained during cytokine challenge in colonized HIOs
**E. coli** colonization enhances epithelial barrier resilience

<table>
<thead>
<tr>
<th>DAPI</th>
<th>PBS</th>
<th><strong>E. coli</strong></th>
<th>TNFα &amp; IFNγ</th>
<th><strong>E. coli</strong> + TNFα &amp; IFNγ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>E-cadherin</strong></td>
<td></td>
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<td></td>
<td></td>
<td><strong>ZO-1</strong></td>
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</tbody>
</table>

Hill *et al.* (2017).
Broad Implications

- Immature epithelium and underlying mesenchyme is sufficient to maintain symbiosis with bacteria
  - Achieved through enhanced epithelial barrier defense
  - No immune cells required

Key questions

Is the epithelial response to primary microbial colonization species-specific?
Designing a screen of representative microbial colonists

**Novel Alternative Model Systems of Enteric Disease (NAMSED)**

**Experimental subsets**

- **Neonatal Commensals/Opportunists**
  - PBS
  - *B. thetaiotaomicron*
  - *E. coli* str. ECOR2
  - *Bifidobacterium infantis*
  - *Lactobacillus reuteri*
  - *Enterobacter cloacae*
  - *Enterococcus faecalis*

- **C. difficile toxin mutants**
  - PBS
  - C. difficile WT
  - C. difficile TdA null
  - C. difficile TcdB null
  - C. difficile TdA & TcdB null

- **Immune Interactions**
  - PBS
  - PBS + PMN
  - *E. coli* str. ECOR2 + PMN
  - *S. typhimurium*
  - *S. typhimurium* + PMN
  - S. typhi
  - S. typhi + PMN

- **Viral pathogenesis**
  - PBS
  - Human norovirus

**Setup culture plates**
- 4-6 HIOs per well
- 1 x 4 well plate per treatment
- 16 to 24 HIOs per treatment

**Microinjection**
- 1 µL per HIO
- Antibiotics in media

**Co-culture (24 h)**
- Δ media following injection
- Maintain antibiotics for 2 h
- Δ to antibiotic-free media

**Collect Samples**

- **HIO**
  - RNA-seq
  - Plate luminal contents to measure bacterial growth

- **Media**
  - ELISA
  - Plate media sample for bacterial CFU

**Immune cells**
- Isolated day of injection
- Added to external media
Robust host transcriptional response to bacterial colonization

RNA-seq differential expression

S. Typhimurium
S. aureus
L. reuteri
E. faecalis
E. coli
E. cloacae
B. thetaiotamicron

log$_2$FC (HIO + bacteria / HIO + PBS)
Identification of strain specific host response gene sets
The host transcriptional response to bacterial colonization is species-specific.
The host transcriptional response to bacterial colonization is species-specific

- **K-means algorithm** finds patterns in a large data set without prior knowledge

- Each gene is assigned to one of $K$ groups based on expression across all colonization conditions

- $K$ is defined *a priori* as part of the hypothesis (*e.g.* There are 7 distinct transcriptional responses to the 7 bacterial colonization conditions)

- Each *cluster* is a list of genes that have a shared pattern across conditions

Hill *et al.* Unpublished data
The host transcriptional response to bacterial colonization is species-specific.
Evidence of specific transcriptional control over genes in each cluster

Over-abundance test: more genes in a list from a given gene set (TFT) than expected by chance.

Hill et al. Unpublished data
Expression of downstream NF-κB targets varies by bacterial strain.
Network analysis reveals novel correlations between gene expression events
What are the functional consequences of variation in epithelial transcriptional response?
Effect of bacteria on epithelial barrier function is highly strain-dependent

Hill et al. Unpublished data
Secreted cytokine response to bacterial colonists varies widely

Hill et al. Unpublished data
Network analysis reveals novel correlations between gene expression events
Epithelial cytokines control AMP secretion
BD-2 inhibits *E. coli* growth *in vitro*
Pre-treatment with IL-1β suppresses microbial growth.
Epithelial cytokines control AMP secretion

**Graph 1:**
- **Y-axis:** BD2 (μg/ml)
- **X-axis:** ng/ml
- **Graph 2:**
- **Y-axis:** BD2 (μg/ml)
- **X-axis:** IL-1β (pg/ml)
- **Legend:**
  - IL-1β
  - IL-1β + IL-17C
- **Statistical Significance:**
  - P = 0.04
  - P = 0.02
Cytokine environment alters epithelial permeability

Dextran permeability ratio (70 kDa/4 kDa)

- IL-17C
- IL-1β
- IL-1β & IL-17C
- PBS

Treatment added

Time (h)
A working hypothesis on bacterial-epithelial interactions during gut colonization

Many questions remain

- How do bacteria elicit distinct epithelial responses?
- How do we distinguish the initial response to bacteria from the response to cytokines induced by bacteria?
- Does the epithelial response to initial colonists shape the environment in ways that affect subsequent colonists?
- Does the epithelial response reflect microbial community composition?
Extending the HIO platform for application to clinical sample sets
Necrotizing enterocolitis is a critical health challenge

- Severe intestinal inflammation and necrosis
- Affects up to 0.5% of US newborns
- 7-fold elevated risk among premature and low birth weight infants
- In-hospital mortality of up to 30%
- Lifelong complications and disability among survivors

There has been no improvement in NEC incidence or mortality in over 40 years.
NEC results from intestinal immaturity and aberrant microbial colonization


NEC results from intestinal immaturity and aberrant microbial colonization

How do we study these processes in human tissues?

Metagenomic Sequencing with Strain-Level Resolution Implicates Uropathogenic *E. coli* in Necrotizing Enterocolitis and Mortality in Preterm Infants

Doyle V. Ward, 1,4,* Matthias Scholz, 2,4 Moreno Zolfo, 2 Diana H. Taft, 3 Kurt R. Schibler, 3 Adrian Tett, 2 Nicola Segata, 2,5 and Ardythe L. Morrow 3,5

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http://dx.doi.org/10.1016/j.celrep.2016.03.015
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Extending the HIO platform for application to clinical sample sets

GOAL: Identify and characterize clinical *E. coli* strains with physiologically relevant effects on the immature intestinal epithelium
### Description of clinical isolates

#### Table 1: Clinical *E. coli* isolates cohort

<table>
<thead>
<tr>
<th>Clinical status</th>
<th>Unique Isolates</th>
<th>DOL* acquisition mean (range)</th>
<th>DOL NEC onset mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-NEC</td>
<td>19</td>
<td>33.7 (8-59)</td>
<td>-</td>
</tr>
<tr>
<td>NEC</td>
<td>28</td>
<td>26.4 (2-58)</td>
<td>31.2 (17-61)</td>
</tr>
<tr>
<td>post-NEC</td>
<td>5</td>
<td>33.3 (20-58)</td>
<td>31.6 (17-56)</td>
</tr>
<tr>
<td>pre-NEC</td>
<td>23</td>
<td>24.9 (2-56)</td>
<td>31.1 (17-61)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>47</strong></td>
<td><strong>29.3 (2-59)</strong></td>
<td><strong>31.2 (17-61)</strong></td>
</tr>
</tbody>
</table>

*Day of Life or days post-partum
Elucidating the mechanistic connections between tissue response, *E. coli* genetics, and clinical outcomes

**GOALS:**
- Evaluate effect of clinical *E. coli* isolates on HIO
- Identify *E. coli* genetic factors that predict the HIO response
Elucidating the mechanistic connections between tissue response, \textit{E. coli} genetics, and clinical outcomes

**GOALS:**
- Evaluate effect of clinical \textit{E. coli} isolates on HIO
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Elucidating the mechanistic connections between tissue response, *E. coli* genetics, and clinical outcomes

**GOALS:**
- Evaluate effect of clinical *E. coli* isolates on HIO
- Identify *E. coli* genetic factors that predict the HIO response
Screening distinct *E. coli* isolates for effects on epithelial barrier permeability in HIOs

**4 kD dextran permeability**

- Isolate 64
- Isolate 75
- PBS

**Median dextran half-life (t½)**

- Isolate 63
- Isolate 64
- Isolate 69
- Isolate 67
- Isolate 72
- Isolate 62
- Isolate 71
- Isolate 58
- Isolate 68
- Isolate 70
- Isolate 66
- Isolate 74
- Isolate 60
- Isolate 76
- Isolate 59
- Isolate 65
- Isolate 73
- Isolate 75
- Isolate 57

**Time post-treatment (h)**

**time (h)**

Dr. Roberto Cieza
Clinical E. coli isolates elicit distinct patterns of cytokine secretion

Median dextran half-life ($t^{1/2}$)

ELISA results

Secreted factor

Dr. Roberto Cieza
Correlating clinical status with HIO response

- **dextran t½ (h):**
  - P = 0.01

- **BD1:**
  - P = 0.04

- **IFNγ:**
  - P = 0.07

- **IL8:**
  - P = 0.01

- **TNFα:**
  - P = 0.05

**Units:**
- **hr:** 0, 25, 50, 75
- **pg/ml:** 0, 200, 400, 600

**Legend:**
- **non-NEC**
- **NEC**
Correlating epithelial barrier permeability with epithelial secretions
A Web of Correlated Data

...and the tools to test those correlations directly

**Ongoing work**

- Sequence, assemble, and annotate genomes for all clinical isolates (Dr. Roberto Cieza)

- Correlate genomic features with HIO response and identify genes associated with barrier function and cytokine secretion

- Test role of specific bacterial and host genes *in vitro* (Molecular Koch’s postulates)

  - Can we identify bacterial genes associated with epithelial barrier integrity?

  - Are these bacterial genes associated with NEC risk in clinical populations?
HIOs are a flexible and modular model system

Microorganism(s)
- Genetics: Reference strains
  - Clinical isolates
  - Genetically engineered
- Products: Metabolites
  - PAMPs
  - Toxins

Microbial communities
- Symbionts
- Pathogens

Organoid
- Source: Tissue
  - iPSCs
  - ESCs
- Genetics: Standardized cell lines
  - Patient genotype
  - Genetically engineered
- Structure: 3D spheroid
  - 2D monolayer

ECM
- Matrix composition
  - Biologic or synthetic origin

Media
- Growth factors
- Cytokines
- Microbial products
- Pharmacological agents
- pH, hypoxia

Hill, DR and Spence, JR. CMGH. 2017
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