Translational Opportunities in the Gut Microbiome

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Humans are Metagenomic Organisms

- The human microbiome exceeds the number of human cells (10 trillion) by at least an order of magnitude.

- Human microbiota encode at least 100-times more genes compared to their host.

- Microbes have co-existed with humans since before we were human.

- Many of these microbial interactions endow or enhance human physiology including processes related to development, nutrition, immunity and resistance to pathogens.

image courtesy of the NIH HMP website http://nihroadmap.nih.gov/hmp/
The Human Microbiome, an Exclusive Club

- **Mouth**
- **Stomach**
- **Colon**
- **Skin**

- Actinobacteria
- Firmicutes
- Proteobacteria
- Bacteroidetes
- Fusobacteria
- Spirochaetes
A consequence of functional redundancy is an increased interpersonal variability of the microbiota.

How does this impact our ability to perform association studies?

Can we learn to recognize functionally synonymous species, viewed as interchangeable?
The Human Microbiota Over the Human Lifespan

- Age 1-2 year
- Stable adult microbiota
- Chronic perturbation (poor diet)
- Inflamm-aging immunosenescence
Understanding the Principles of Microbiota Resilience

Vaginal

C-section

Highly adapted community

Time (days)

1 30 60 90 120 180 240 300 360 420 480 540 600 660 730

Table food: 1 7 14

Solid food: 1 7 14

Tooth eruption: 1 3 5

Antibiotic exposure: 1 7 14
Microbiota Assembly: In Search of Stable Configurations
Mouse Model of Aging

Peterson and Baaten, SBMRI
The Far Reach of the Gut Microbiota

Hypothesis: Transplantation of young microbiota into aged mice protects against infection and reverses chronic inflammation (inflammaging).
Communication at a distance is good and leads to inflammatory tone.

Commensal bacteria are kept at a distance by a thick 150 \( \mu \text{m} \) mucin layer.

IgA, \( \alpha \)- and \( \beta \)-defensins and C-lectin, RegIII\( \gamma \) serve to keep the proximity of gut microbes in check and at a healthy distance.

DC regularly sample commensal bacteria through the epithelium and “primed” by live bacteria.

The inevitable instances of epithelial breach are aggressively engulfed by macrophage and DCs.
Dysbiosis: The numerically dominant Bacteroidetes express a pentacylated LPS (poor TLR4 agonist) whereas the far less abundant Proteobacteria express a hexacylated LPS (potent agonist). Shifts in this relationship can lead to chronic inflammatory signals and IBD.
The Obesity Epidemic in the USA

Obese and overweight individuals are at increased risk of developing type II diabetes, hypertension, heart disease, stroke, non-alcoholic fatty liver disease, osteoarthritis, depression, liver and colorectal cancers. It is predicted that by 2030, 42% of the US population will be obese resulting in increases in healthcare spending by $550 billion.
Mannose Induces a Lean Phenotype in Mice

Graphs showing weight gain over age for different dietary conditions:

(a) Mannose
- ND
- HFD
- HFD+M
- ND+M

(b) Galactose
- ND
- HFD
- HFD+gal

(c) Mannose removed
- ND
- HFD
- HFD+M

(d) 3 weeks post weaning
- ND
- HFD
- HFD+M

Legend:
- * p < 0.05
- ** p < 0.01
- *** p < 0.001
- **** p < 0.0001
- ns = not significant

Sample sizes:
- N = 14-20
- N = 4
- N = 8

Note: Mannose Induces a Lean Phenotype in Mice
Mannose Reduces High Fat Diet-induced Adiposity

(a) 45% HFD

(b) 45% HFD, mannose started 3 weeks post-weaning

(c) 60% HFD

(d) Epididymal adipocytes

\(N = 5\) for 60% HFD

\(N = 8\) for 45% HFD, mannose started 3 weeks post-weaning

** Mannose reduces high fat diet-induced adiposity
Mannose Has Nominal Impact on Blood Parameters

<table>
<thead>
<tr>
<th>Table 1. Blood serum parameters (n=8).</th>
<th>Serum</th>
<th>ND</th>
<th>HFD</th>
<th>HFD+M</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>245±10</td>
<td>277±9</td>
<td>238±13</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>88±4</td>
<td>71±13</td>
<td>57±3</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>free fatty acids µM</td>
<td>827±29</td>
<td>811±27</td>
<td>771±16</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>Glycerol (mg/dL)</td>
<td>5.1±.2</td>
<td>5.2±.3</td>
<td>4.3±.2</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>total cholesterol (mg/dL)</td>
<td>132±7</td>
<td>155±5</td>
<td>189±3</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>VLDL/LDL (mg/dL)</td>
<td>23±2</td>
<td>26±2</td>
<td>27±2</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>105±6</td>
<td>116±10</td>
<td>151±7</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>insulin ng/mL</td>
<td>1.3±0.3</td>
<td>2.4±.6</td>
<td>1.3±.2</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>adiponectin µg/mL</td>
<td>22±1</td>
<td>31±2</td>
<td>34±3</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>leptin (ng/mL)</td>
<td>2.3±.4</td>
<td>6.5±1.0</td>
<td>4.6±1.2</td>
<td></td>
<td>ns</td>
</tr>
</tbody>
</table>
Mannose Improves Glucose Tolerance and Insulin Sensitivity

(a) GTT

(b) ITT

AUC above baseline (mg/dL)X90 min

slope of glucose fall (mg/dL)X30 min

ND HFD HFD-M

** * ns *
Mice Supplemented with Mannose Consume More Calories

(a) 45% HFD

- HFD
- HFD+M

Cumulative food intake (g)

Time (hours)

n = 6

(b) 60% HFD

- HFD
- HFD+M

Cumulative food intake (g)

Time (hours)

n = 5
Mannose Alters the Composition of the Gut Microbiota

(a) 120%

ND HFD HFD+M

(b) 120%

Mannose removed at 16 weeks

HFD HFD+M/-M

- Deferrribacteres
- Cyanobacteria
- Synergistetes
- Actinobacteria
- Verrucomicrobia
- Tenericutes
- Proteobacteria
- Firmicutes
- Bacteroidetes

* **** ***

%
Mannose Induces Changes in Microbiota Gene Expression

- Methanobrevibacter
- Lactococcus
- Faecalibacterium
- Bifidobacterium
- Odoribacter
- Akkermansia
- Solobacterium
- Bacteroidetes
- Bacteroides
- Lactobacillus

Legend:
- ND
- ND+M
- HFD
- HFD+M
Energy Harvest

Mannose induced decreased expression (~50%) of: Glu-1-P-adenyltransferase, involved in starch metabolism.

1,4-α-glucan debranching, enzymes involved in β-D-glucose liberation from starch.

α-amylase, endoglucanases, and endoglycosidases.
## Energy Content and SCFA Production

<table>
<thead>
<tr>
<th>Mice n=7-8</th>
<th>HFD</th>
<th>HFD+M</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannose (µmol/g)</td>
<td>0.15±0.6</td>
<td>5.9±1.6</td>
<td>**</td>
</tr>
<tr>
<td>SCFA (µmol/g)</td>
<td>3.3±0.4</td>
<td>7.6±1.6</td>
<td>*</td>
</tr>
<tr>
<td>Acetate (µmol/g)</td>
<td>2.1±0.4</td>
<td>5.9±1.4</td>
<td>*</td>
</tr>
<tr>
<td>Propionate (µmol/g)</td>
<td>0.26±.04</td>
<td>0.49±0.1</td>
<td>*</td>
</tr>
<tr>
<td>Butyrate (µmol/g)</td>
<td>0.18±.01</td>
<td>0.45±.08</td>
<td>**</td>
</tr>
</tbody>
</table>

### Fecal energy content

![Fecal energy content graph](image)
Flagellins Induce Inflammation via TLR5 Signaling

Gut mucosal inflammation
Altered luminal flora / bacterial overgrowth
Endotoxemia / flagellin / cytokinemia
Parenteral nutrition / lack of enteral feeding
Protein-energy malnutrition

Luminal microbes

Adherens junction

Tight junction

TLR5

NF-κB

IL-8

Innate immune response

PMNs

Flagellin

Dendritic cell

T-cell

Adaptive immune response

B-cell

Flagellin-specific IgM, IgA, IgG
Indirect Evidence Linking Gut Microbiota to CRC

The density of bacteria in the small intestine ileum ($10^{2-3}$/mL) increases substantially along the length of the intestine and is highest in the colon ($10^{12}$/mL), corresponding to an ~12-fold increased prevalence of colon tumors.

Germ-free mice develop fewer colon tumors compared to conventional mice.

Infections by specific pathogens, e.g. *S. gallolyticus* have been associated with CRC. Colonization by enterotoxin-producing *B. fragilis* (ETBF), *E. coli*, *Enterococcus* spp. and *Enterobacteria* spp. produce genotoxic and inflammatory molecules and promote CRC.

Human subjects with inflammatory bowel disease (IBD) display gut microbiota dysbiosis and have a 5-fold increased risk of developing CRC.
These results indicate that ETBF promotes colon tumorigenesis via activation of Th17 cell responses.
E. coli

**F. nucleatum**

**A**

<table>
<thead>
<tr>
<th>Macrophages</th>
<th>Correlation with <em>Fusobacterium</em> abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD209</td>
<td>0.290 (Spearman) 0.0343 (corrected)</td>
</tr>
<tr>
<td>MRC1 (CD206)</td>
<td>0.269 (Spearman) 0.0446 (corrected)</td>
</tr>
<tr>
<td>IL6</td>
<td>0.419 (Spearman) 0.0007 (corrected)</td>
</tr>
<tr>
<td>IL8</td>
<td>0.473 (Spearman) 0.0007 (corrected)</td>
</tr>
<tr>
<td>CXCL10</td>
<td>0.261 (Spearman) 0.0499 (corrected)</td>
</tr>
</tbody>
</table>

**B**

<table>
<thead>
<tr>
<th>GO Biological Function</th>
<th>Corrected P-val</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory response</td>
<td>2.0x10^{-14}</td>
</tr>
<tr>
<td>Cell-cell signaling &amp; interact.</td>
<td>2.0x10^{-12}</td>
</tr>
<tr>
<td>Immune cell trafficking</td>
<td>2.4x10^{-14}</td>
</tr>
<tr>
<td>Cellular growth &amp; proliferation</td>
<td>1.1x10^{-14}</td>
</tr>
<tr>
<td>Cell death</td>
<td>1.3x10^{-11}</td>
</tr>
<tr>
<td>Immunological disease</td>
<td>3.1x10^{-17}</td>
</tr>
<tr>
<td>Inflammatory disease</td>
<td>3.1x10^{-17}</td>
</tr>
<tr>
<td>Lymphoid tissue struct. &amp; dev.</td>
<td>5.5x10^{-16}</td>
</tr>
</tbody>
</table>

**C**

*Fusobacterium*-associated host gene expression signature

**D**

<table>
<thead>
<tr>
<th><em>Fusobacterium</em> high</th>
<th><em>Fusobacterium</em> low</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-κB p65</td>
<td>lamin B1</td>
</tr>
<tr>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>80</td>
<td>60</td>
</tr>
</tbody>
</table>

**E**

*Mouse ApcMin/+* model

- **II1β**
  - P = 0.02
  - Shown in Colon tumors, S. I. tumors
- **II6**
  - P = 0.014
  - Shown in Colon tumors, S. I. tumors
- **IL24**
  - P = 0.028
  - Shown in Colon tumors, S. I. tumors
- **Mmp3**
  - P = 0.031
  - Shown in Colon tumors, S. I. tumors
- **Scyb1 (IL8)**
  - P = 0.015
  - Shown in Colon tumors, S. I. tumors
  - P = 0.011
  - Shown in Colon tumors, S. I. tumors
  - P = 0.075
  - Shown in Colon tumors, S. I. tumors
Driver-Passenger Model of Microbiota-Induced Colorectal Cancer

Tjalsma, et al., Nature Reviews Microbiology 10, p.575
Right Sided Tumors are Associated with Bacterial Biofilms

50 human subjects: 48% of all tumors (15/31) and 67% of adenomas (4/6). 87% (13/15) tumors and 100% (4/4) adenomas on right-sided CRC. Transverse and descending tumors 13% (2/16) and 0% biofilms (0/2).
Paired CRC Samples

- Bacteroidaceae
- Comamonadaceae
- Leuconostocaceae
- Porphyromonadaceae
- Prevotellaceae
- Rikenellaceae
- Burkholderiaceae
- Incertae Sedis XI
- Lachnospiraceae
- Ruminococcaceae
- Veillonellaceae
- Enterobactericeae
- Fusobactericeae
- Streptococcaceae

Paired CRC Samples
Normal Adjacent Tissue Has Hallmarks of Premalignancy

A

Biofilm positive normal mucosa from CRC patient A

Biofilm negative normal mucosa from CRC patient B

E-cadherin

IL-6

P-STAT3

B

Biofilm positive normal mucosa from subject without CRC

Biofilm negative normal mucosa from subject without CRC

C

E-cadherin mean fluorescence intensity (A.U.)

Paired normal Biofilm

Paired normal No Biofilm

p<0.03

D

IL-6 epithelial cell fluorescence intensity (A.U.)

Paired normal Biofilm

Paired normal No Biofilm

p<0.001

E

CRC IL-6 (pg/ml)

Paired normal Biofilm

Paired normal No Biofilm

p<0.03

F

Epithelial cell P-STAT3 IHC Score

Paired normal Biofilm

Paired normal No Biofilm

p<0.04
126 paired samples analyzed by 16S rDNA profiling.
Phylum level alterations

**Bacteroidetes**

- Healthy: <0.0001
- Normal: <0.0001
- Tumor: <0.0001

**Firmicutes**

**Proteobacteria**

**Firm-Bact ratio**

- Healthy: 0.06
- Normal: 0.01
- Tumor: 0.01
Dysbiosis

**Bacteroides fragilis**

- Normal: 0.0002
- Tumor: 0.0002

**Bacteroides propionicifaciens**

- Normal: 0.0012
- Tumor: 0.0012

**Faecalibacterium prausnitzii**

- Normal: 0.0093
- Tumor: 0.0093

**Fusobacterium nucleatum**

- Normal: 0.0262
- Tumor: 0.0262
Following Up

Escherichia coli

Burkholderiaceae; Pandoraea

Firm-Bact ratio
Organoid Culture

Niche factors
- Wnt
- EGF
- Noggin
- R-spondin
- ALK4/5/7 inhibitor
- p38 inhibitor

Niche factors
- Various requirements:
  - EGF
  - Noggin
  - ALK4/5/7 inhibitor
  - p38 inhibitor

Matrigel

Normal Epithelium

Tumor Epithelium
IgA-Seq

A

Fecal Pellet
Homogenize
Stain with Anti-IgA
Enrich IgA+ by MACS
Sort IgA+ by FACS
16s rRNA sequencing

B

IgA+

Total

IgA-

IgA
Thanks for your attention

Questions?

SBMRI
Manuel Perucho
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Vandana Sharma
Alex Strongin
Carl Ware
Bas Baaten

Nik Schork (JCVI)
Cynthia Sears (JHU)