The Microbiome: A New Lens for Human Disease

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Overview

• Human Microbiome Project
  1. Nasal Microbiota
  2. Pulmonary Microbiota
  3. Intestinal Microbiota

• Pharmaceutical Control of the Microbiome

Human Microbiome Project

• 242 healthy US adults
• Sampled 3x from 15-18 body sites
• 5,177 bacterial taxa via 16S rRNA sequencing
• 3.5 Tbp metagenomic sequencing data
• Assembled sequences for 800 bacterial reference strains


HMP Overarching Conclusions

• Skin: greatest bacterial diversity
• GI: greatest bacterial load
• Mucosa: highly specialized and complex commensal communities
  – Diversity typically associated with health
  – Lack of diversity (dysbiosis) associated with disease

Microbia and the Mucosa: Intimate Symbiosis
Nasal Microbiota & S. aureus

- Normal: Actinobacteria, Firmicutes
- Hospitalized: Decreased Actinobacteria, increased S. aureus, S. epidermidis
- S. aureus outcompetes S. epidermidis

Corynebacterium accolens & pseudodiptheriticum co-localized with S. aureus
- Assisted S. aureus growth in co-culture

Nasal Microbiota & Rhinosinusitis

- Same microbiota in normal and rhinosinusitis patients
- Hyerpactive immune responses in rhinosinusitis

- Decreased lactic acid bacteria and increase in Corynebacterium tuberulostearicaum in rhinosinusitis
  - Lactobacillus sakei decreased sinus infections by C. tuberulostearicaum

Lung Microbiota & Cystic Fibrosis

- CF associated with increased Haemophilus influenzae, S. aureus, Pseudomonas aeruginosa, Burkholderia cepacia, along with Prevotella, Streptococci, Rhothia, Viellonella
- Lower diversity, lower lung function

- Soil Burkholderia dolosa marker for CF outbreaks
  - Adaptive mutations mapped over 15+ years

AIR
microbiota
Lungs

Plasma

epithelial mucosa

0.018 m²

Nasal – Sinus

NASAL – SINUS

Lung

Plasma

epithelial mucosa

70-100 m²

LUNG

AIR

microbiota

LOWER

UPPER
Lung Microbiota & COPD

- COPD: increased *H. influenzae* and Proteobacteria after viral challenge
- Persists for >40 days post viral infection


Lung Microbiota & Asthma

- Farm children: more bacteria and bacterial diversity, less asthma
- Microbial diversity at key stages of development tunes immune tolerance
- Changing GI composition changes short-chain fatty acid metabolism, which is protective against allergic asthma
  - SCFA: more pulmonary DC to control allergic inflammation

*NEJM* (2011) 364: 701

GI Microbiota, Diet & Obesity

- Diet changes GI microbiota
- Reduced microbiota diversity, increased carbohydrate metabolism lead to obesity in one twin
- Early antibiotics change microbial diversity and fat mass in mice
- Competition in virulence and energy metabolism drive GI microbial pathogenesis


GI Microbiota & Atherosclerosis

- TMAO is risk marker for atherosclerosis and generated by choline, phosphatidylcholine metabolism and the GI microbiota
- L-carnitine from red meat metabolized by GI microbiota to TMAO
- TMAO may inhibit reverse cholesterol transport

*NEJM* (2013) 368: 1575
**GI Microbiota, Crohn’s & UC**

- Disruptions of innate and adaptive immunity linked to inflammatory Crohn’s and ulcerative colitis (and even colorectal cancer etiology)
- Microbiota “set the tone” for proper immunity in the GI
- *Bacteroides fragilis* produce specific α-galactosylceramide that agonizes nKT-cells
- Microbial signals keep GI-proximal immune cells in “good working order”

*Cell Microbiol.* (2014) 16: 1053

**GI Microbiota & Drug Actions**

- Digoxin inactivated by GI microbe *Eggerthella lenta*
- Anticancer CpG oligos and Pt drugs require GI microbiota to drive tumor-infiltrating myeloid-derived cells to deliver ROS
  *Science* (2013) 342: 967
- Anticancer cyclophosphamides required GI bacteria to infiltrate lymphoid tissue to stimulate immune cell attack
  *Science* (2013) 342: 971
- Irinotecan & NSAID toxicity...

**GI Microbiota**

**FOOD**

- Diet, Obesity
- Specific Gene Expression
- Pathogen Exclusion
- Altered Diversity

**Drug Actions**

- Altered Diversity
- Specific Enzymes
- Immune Activation
- Targeted Modulation

**Neurological Disorders**
- Diabetes
- Autoimmune
- Heart Disease
- Cancer
- Inflammation
- Drug Toxicity

**GI Microbiota & Neurology**

- 100s Species
- Millions Genes
- Trillions Cells

**Pharmaceutical Targets**

**GI Microbiota & Carboxylesterases (CE)**

- Alleviation of GI Toxicity Caused by Anticancer Drug Irinotecan
**β-Glucuronidase**

**Human**
- Large glucosaminoglycans
- Sly Syndrome
- Essential

**Bacterial**
- Small glucuronate ethers
- Sugar scavenging
- Non-essential

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**GOAL:** Inhibit Bacterial $\beta$-Glucuronidases

![Diagram of GOAL: Inhibit Bacterial $\beta$-Glucuronidases](image)

- Potent
- Selective
- Non-Lethal

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**Novel Inhibitors by HTS**


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<th>$K_i$ (nM)</th>
<th>$K_{IC50}$ (nM)</th>
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$K_i = 160 - 680$ nM

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**E. coli β-glucuronidase**

2.3 Å resolution inhibitor 2 complex
2.4 Å resolution inhibitor 3 complex

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**Selective**

**GOAL**: Inhibit Bacterial β-Glucuronidases

- Potent
- Selective
- Non-Lethal

**Protection from CPT-11 Toxicity**

- Bacterial β-Glucuronidase Inhibitor
- 10 μg 2 x Daily Oral Gavage

**No Impact on CPT-11 Plasma PK**

- Measured Concentration (ng/mL)
- Hours

**Drug-Induced Toxicity**

1. Anticancer Drug CPT-11
2. NSAIDs

**NSAID Toxicity**

- Diclofenac-glucuronide
- Anti-inflammatory efficacy
- Ulcers
- Small GI

**Small Intestinal Toxicity**

- Long-Term Human NSAID Users
  - 70% INFLAMMATION
  - INCREASED PERMEABILITY
  - MALABSORPTION
  - 40% ULCERS
  - 30% BLEEDING, ANEMIA
Alleviation of NSAID Toxicity?

Protection from Diclofenac Toxicity

Protection from Diclofenac Toxicity

No Impact on Diclofenac Plasma PK

Drug-Induced Toxicity

Pharmaceutical Microbiome Control
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