Gut Microbiome
Objectives

• Understand the microbial ecology of the gut

• Appreciate the role of the human intestinal microbiota in both normal and abnormal gut and systemic function

• Explore various disorders associated with microbiome alterations

• Assess the possible impact of modulating the bacterial milieu in various disease states
Gut Microbiota as a Regulator of Health

- Metchnikoff and Mitchell in 1907
  - The prolongation of life: optimistic studies
  - Hypothesized that toxins produced by putrefactive colonic microbes accelerate senescence

- Large consumption of fermented milk in certain eastern European rural populations famed for purported longevity

- Metchnikoff introduced sour milk into his own diet and noticed improvement in his own health, forming the foundation for probiotics

Borody and Khoruts *Nat Rev Gastroenterol Hepatol* 2014
Human Microbiome

• Increased interest recently

• Of over 5,000 articles, about 90% published in the last 5 years

• Growing realization of the role that our commensal microbiota plays in human health and disease

• Human gut microbiota is a largely unexplored ecosystem
Human body contains trillions of microbial cells (microbiota)

- Most within gut (100 trillion)
- Bacteria (Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria)
- Archaea, Viruses, Fungi

Aggregate microbial genome (microbiome) contains >100 fold more genes than human genome (gene transfer)

Crucial to our health and well-being

Modified from DiBaise
Microbiota Development and Ecology

>1,000 Microbial Species

AEROBES

Stomach
<10^2 cfu/mL
pH 1-2

Duodenum
10^{1-3} cfu/mL
pH 6-7

Jejunum
10^{3-4} cfu/mL
pH 6-7

Ileum
10^{7-9} cfu/mL
pH 6-7

Colon
10^{10-12} cfu/mL
pH 5-7

ANAEROBES

WHAT IS KNOWN

- Colonization begins at birth
- Develops in early life (feeding and play)
- Unique to each individual
- General stability throughout life
- Affected by internal and external factors
- Alterations in disease

Modified from DiBaise MCP 2008
Actions of the Gut Microbiota

- Intestinal development
- Epithelial turnover, motility, blood flow
- Immune system development/modulation
- Energy utilization and storage
- Micronutrients and SCFA
- Block colonization with pathogenic bacteria
- Drug metabolism

Modified from Parkes *Am J Gastroenterol* 2008
Microbiome is Influenced by Multiple Factors

Healthy Status

- Host genetics: Mutations in NOD2, IL23R, ATG16L and IGRM
- Lifestyle: Diet, Stress
- Medical practices: Vaccination use, Antibiotic hygiene
- Other: Maternal vertical transmission, GI infections

Modified from Kashyap *World Summit 2014*
Diversity in gut bacterial communities of school-age children in Asia

Prolonged use of a PPI reduces microbial diversity
Implications for *Clostridium difficile* susceptibility

- Observational studies associate PPI use and CDI incidence
- 9 healthy and 5 treatment-naïve CDI subjects
- Random assignment to PPI for 28 days
- Stool microbiome samples collected/analyzed/compared
- PPI usage
  - Decreased operational taxonomic unit (OTU) counts
  - Similar to treatment-naïve CDI group
- Partly reversible after a 1-month drug withdrawal

Intestinal Microbiota

• Like a postnatal acquired organ that performs different functions for the host

• Maximal diversity during adolescence

• Remains stable until later stages of life, where the microbiota becomes comparatively less diverse with reduced stability (associated with “inflamm-ageing”)

• Lack of diversity, dysbiosis associated with disease states
Hygiene hypothesis
• Antibiotic overuse
• Lifestyle changes
  — Diet
  — Exercise
• Aging

Microbiome Assault Over the Past Six Decades
Explanations for Increase in Human Disease

Courtesy John DiBaise
Potential Role in Human Disease

Gut Microbiota

- Metabolic syndrome
- Cardiovascular diseases
- Endotoxemia/septicemia
- Rheumatoid arthritis
- NAFLD/NASH/HS
- Hypertension
- Endocrinial imbalance
- Asthma
- Colorectal cancers
- Diarrhea/constipation/Celiac disease/Gastroenteritis
- Diabetes/Insulin resistance
- Obesity/Adiposity
- IBD/IBS/UC/Crohn’s disease

Nagpal Front Med 2014
**Clostridium difficile Infection (CDI)**

- Affects > 1 million yearly (United States)
- Risk factors – ↑age, antibiotic exposure, hospitalization, severe comorbidities, IBD, malignancy, chemotherapy
- Above associated with decreased gut microbial diversity
- CDI recurrence involves ongoing disruption of the normal gut microbiota and inadequate host immune response
- Recurrent CDI
  - 1st case  \(20-30\%\)
  - 3 or more infections \(\geq 60\%\)
- Antibiotics may further disrupt colonic microbial communities, resistant spores
CDI
Pseudomembranous Colitis

Normal colonic mucosa

Pseudomembranes
Gut Microbiota is Altered in Patients with IBS

* Decreased diversity and Bacteroidetes
Gut Microbiota and Mechanisms in IBS

Gut microbiota

Altered permeability

Visceral hypersensitivity

Altered gastrointestinal motility

Neurotransmitter release

Brain gut axis
Inflammatory Bowel Disease

• Gut microbiota has a major role in barrier function and immune regulation

• Mice don’t develop colitis in a germ-free environment

• Gut microbiota findings in IBD
  — Reduced species diversity
  — Lower temporal stability
  — Structural disruption of the secreted mucous layer in IBD patients

• Cause versus effect

• Fecal diversion, antibiotics, probiotics
Altered Duodenal Microbiota Composition in Celiac Disease Patients Suffering From Persistent Symptoms on a Long-Term Gluten-Free Diet

Wacklin, et al. AJG 2014
Gut Dysbiosis and Metabolic Disease States

- Metabolic syndrome
  - Healthy state
    - Normal microbiota
  - Diseased state
    - Fewer Bacteroidetes phylotypes
    - Fewer Lachnospiraceae phylotypes

- Type 1 diabetes
  - Diseased state
    - Typical NOD mouse microbiota
  - Protected state
    - More Porphyromonadaceae phylotypes
    - More Lactobacillaceae phylotypes
    - More Rikenellaceae phylotypes

Gut Microbiota and Obesity

- Fermentation of indigestible dietary polysaccharides
  - Increased intestinal absorption of monosaccharides and short-chain fatty acids
  - Increased hepatic lipogenesis via ChREBP and SREBP-1

- Increased LPL activity via suppression of Fiaf and induction of PGC-1α and AMPK activity
- Increased circulation of LPS modulated by dietary fat content

Increased fatty acid metabolism and storage of calories in fat
Colon Cancer and the Microbiota

• Tumor tissue from colon cancers has less microbial diversity

• Long-term and repeated antibiotic exposure might increase colorectal cancer risk

• Biofilm associated with colorectal cancer
  • 89% (13/15) of right-sided tumors
  • 12% (2/15) of left-sided tumors
  • None of the normal mucosa
  • Associated with a reduced or redistributed colonic epithelial cell E-cadherin
  • Increased permeability → direct access of bacterial antigens/mutagens → promote procarcinogenic tissue inflammation
Allergic Diseases

• Prevalence increased over last several decades

• Asthma, eczema, food allergies

• Hygiene hypothesis – microbial exposures during childhood are crucial to the development of the immune system

• Developmental alterations predispose to loss of self-tolerance

• Altered microbial colonization (perinatal, early childhood; especially recurrent antibiotics) → dysregulated immune responses → allergic and atopic conditions
Rheumatoid Arthritis and Autoimmunity
Brisca et al *Curr Opin Rheumatol* 2014

- Genes are insufficient to explain rheumatoid arthritis

- Presence of bacteria in mucosal surfaces alter local and systemic host immune responses and elicit joint inflammation

- Theory of *bacteria* sharing similar *pro-inflammatory* properties serving as a *trigger* in various mucosal sites in genetically predisposed individuals
  - Peridontal disease strongly linked to RA
  - Gut microbiome altered in mouse models of inflammatory arthritis and human patients with RA
  - Airway inflammation and autoimmunity present at preclinical stages
Gut bacteria/bacterial products can influence CNS function

Altered blood brain barrier

Altered gut microbiota

Binding to cross-reactive epitopes

Collins et al. Nature Reviews Microbiology 2012

Hornig Curr Opinion Rheumatol 2013
Is there a role for modulation of the bacterial milieu in gastrointestinal or other disorders?
Potential Interventions
Microbiota restorative therapies

- Diet modification
- Antibiotics
- Prebiotics, Probiotics and Synbiotics
- Fecal microbiota transplantation
- Bariatric surgery
Long Term and Short Term Dietary Effects on Gut Microbiome

Wu et al. Science 2011
David et al. Nature 2013
Rifaximin vs. Placebo for Non C-IBS

Relief from IBS Symptoms

Global IBS Symptoms

Change in Daily IBS Score

* 2 week treatment and 10 week post-treatment period

Probiotics and Fecal Microbiota Transplant (FMT)
Probitoics

- Live organisms that confer potential health benefits
- Wide use in Europe and Asia
- Increasing popularity in US
- Interest in scientific credibility
- Easily available without a prescription
- Proposed use in inflammatory, infectious, neoplastic, allergic GI and non-GI conditions
Proposed Probiotic Mechanisms

1. Physical barrier
2. Altered epithelial surface glycosylation pattern
3. Increased mucus production
4. Secretion of antimicrobial peptides
5. Modulation of the immune system

Borowiec & Fedorak, Curr Gastroenterol Rep 2007
# Recommendations for Probiotic Use
Modified from Floch *J Clin Gastroenterol* 2011

<table>
<thead>
<tr>
<th>CLINICAL CONDITION</th>
<th>EFFECTIVENESS</th>
<th>ORGANISM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Acute infection</td>
<td>A</td>
<td><em>S. boulardii, Lactobacillus GG, L. reuteri SD2112</em></td>
</tr>
<tr>
<td>• Prevention of infection</td>
<td>B</td>
<td><em>S. boulardii, LGG</em></td>
</tr>
<tr>
<td>• Ab-assoc (prevention)</td>
<td>A</td>
<td>*S. boulardii, LGG, combination of <em>L. casei</em>, <em>L. bulgaricus</em> and <em>S. thermophilus</em></td>
</tr>
<tr>
<td>• C. difficile (treat/prevent)</td>
<td>B/C</td>
<td><em>S. boulardii, LGG, bacteriotherapy</em></td>
</tr>
<tr>
<td>IBD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Crohn’s disease</td>
<td>C</td>
<td><em>E. coli Nissle, S. boulardii, LGG</em></td>
</tr>
<tr>
<td>• UC (induce rem/maintain)</td>
<td>B/A</td>
<td><em>E. coli Nissle, VSL#3</em></td>
</tr>
<tr>
<td>• Pouchitis (prevent/induce)</td>
<td>A/C</td>
<td>VSL#3</td>
</tr>
<tr>
<td>IBS</td>
<td>B/C</td>
<td><em>B. infantis, VSL#3; B. animalis, L. plantarum</em></td>
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# Recommendations for Probiotic Use

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<tr>
<td>Immune Response</td>
<td>A</td>
<td>LGG, <em>L. acidophilus LAFT1</em>, <em>L. plantarum</em>, <em>B. lactis</em>, <em>L. johnsonii</em></td>
</tr>
<tr>
<td>Allergy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atopic exema associated with cow’s milk allergy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Treatment</td>
<td>A</td>
<td>LGG, <em>Bifidobacterium lactis</em></td>
</tr>
<tr>
<td>• Prevention</td>
<td>A</td>
<td>LGG, <em>Bifidobacterium lactis</em></td>
</tr>
<tr>
<td>Radiation enteritis</td>
<td>C</td>
<td>VSL#3, <em>L. acidophilus</em></td>
</tr>
<tr>
<td>Vaginosis and vaginitis</td>
<td>C</td>
<td><em>L. acidophilus, L. rhamnosus GR-1, L. reuteri RC14</em></td>
</tr>
</tbody>
</table>
Probiotic Use
Has our enthusiasm outpaced the science?

• Several probiotic preparations show promise in various conditions (~20 organisms used)
• Most studies have important methodologic limitations
• Difficult to assess significance/applicability
• Preparations are different in composition, dose, biologic activity (strain-specific outcomes)
• No preparation is FDA-approved
• Most probiotics not covered by insurance carriers
• Cost to be considered
FMT – The Origins

• China, 4th century AD
• Physician Ge Hong described fecal solutions for the treatment of food poisoning and severe diarrhea

• Ming dynasty of the 16th century AD
• Li Shizhen used fecal solution for treatment of abdominal diseases with severe diarrhea, fever, pain, vomiting
• Called “yellow soup”
Microbiota Restoration with FMT

- Transfer gut microbiota from a healthy donor into a recipient
- Goal is engraftment/re-establishment of a normal, stable microbial community within the gut
- Eiseman and colleagues treated 4 CDI patients in the 1950s*
- Arguably the most effective method in treating recalcitrant CDI
- Becoming more widely available

* Eiseman et al. *Surgery* 1958
Clinical resolution rates of rCDI with FMT

- Garborg et al., 2000: 0.83 (0.67, 0.93)
- MacConnachie et al., 2009: 0.73 (0.45, 0.92)
- Polak et al., 2011: 0.87 (0.60, 0.98)
- Lund-Tonnesen et al., 1998: 0.83 (0.59, 0.96)
- Kassam et al., 2010: 0.93 (0.76, 0.99)
- Kelly et al., 2012: 0.92 (0.75, 0.99)
- Mellow and Kanatzar, 2011: 0.92 (0.64, 1.00)
- Mattila et al., 2012: 0.94 (0.86, 0.98)
- Rohlke et al., 2010: 1.00 (0.82, 1.00)
- Yoon and Brandt, 2010: 1.00 (0.74, 1.00)
- Aas et al., 2003: 0.83 (0.59, 0.96)
- Combined: 0.89 (0.84, 0.93)

90% patients with clinical resolution (245/273 pts)
Duodenal Infusion of Donor Feces for Recurrent
Clostridium difficile

Els van Nood, M.D., Anne Vrieze, M.D., Max Nieuwdorp, M.D., Ph.D., Susana Fuentes, Ph.D., Erwin G. Zoetendal, Ph.D., Willem M. de Vos, Ph.D., Caroline E. Visser, M.D., Ph.D., Ed J. Kuijper, M.D., Ph.D., Joep F.W.M. Bartelsman, M.D., Jan G.P. Tijssen, Ph.D., Peter Speelman, M.D., Ph.D., Marcel G.W. Dijkgraaf, Ph.D., and Josbert J. Keller, M.D., Ph.D.
Duodenal infusion of FMT for rCDI

van Nood, et al. NEJM 2013
Process for FMT at MCF
Recipient Screening

• **Inclusion criteria for recipient**
  - 3rd or greater episode of *C. difficile* infection
  - Proven by a positive *C. difficile* stool assay
  - Previous treatment with 1st line therapies for *C. difficile* infection (vancomycin, metronidazole, or fidaxomicin)
  - Previous receipt and failure of at least 1 course of a 6-8 week vancomycin taper or vancomycin treatment followed by rifaximin chaser for 2 weeks.
  - Refractory moderate to severe *C. difficile* diarrhea, failing vancomycin therapy after >1 week
  - Able to safely undergo and consent to colonoscopy
  - Able to identify potential donor and pay for donor screening – *family member / household contact* is preferred
  - Ability to stop gastric acid suppression (at least 7 days prior procedure) medications and concomitant antibiotics

• **Exclusion criteria for recipient**
  - Severe bowel disease precluding colonoscopy
  - Severe underlying immunosuppression
  - Decompensated liver cirrhosis
  - Severely ill ICU patients
Donor Screening

- Family member/household contact is preferred

- **Exclusion criteria for donor**
  - Active communicable illness (HIV, HBV, HAV, HCV)
  - Metabolic syndrome or an autoimmune disorder
  - Recent or chronic diarrheal disorder
  - Irritable bowel syndrome, chronic constipation or diarrhea
  - Inflammatory bowel disease
  - Known colonic GI malignancy or polyposis syndromes
  - High risk sexual behavior (Men having sex with men, HIV, Multiple partners)
  - Illicit drug use; recent tattoos or incarceration
  - Exposure risk for hepatitis or HIV in the past 12 months
  - Travel to high risk areas for infectious diarrhea in past 6 months
  - High risk for Creutzfeldt-Jakob disease
  - History of *C. difficile* infection
  - Hospitalization within the past 3 months
  - Antibiotics within the last 3 months
  - Immunosuppressive or antineoplastic medication use
  - Fever of unknown origin or any suspected infectious disease
Day of FMT

• Fresh donor sample (room temperature) is collected within six hours of scheduled procedure
• Brought to the lab by the recipient or donor the afternoon of procedure
• Sample is processed
• Recipient is prepped as for typical colonoscopy
• Colonoscopy is performed and fecal material instilled in terminal ileum and cecum
• Follow up phone call to patient 1, 3, 6 months
FDA regulations on FMT for rCDI

Fall 2012: Feces considered a drug

June 2013: “enforcement discretion”

FMT for any non-rCDI indication requires an IND

1. Adequate informed consent from the patient.
2. Donor is known to either the patient or the treating health care provider.
3. The stool donor and stool are qualified by screening and testing performed under the direction of the health care provider for the purpose of providing the FMT product to treat his or her patient.
Dysbiosis and Disease

More studies with FMT

- Ulcerative colitis
- Crohn’s disease
- IBS
- Chronic constipation
- Obesity
- Diabetes mellitus
- Multiple sclerosis
- Parkinson disease
- Atopy
- Rheumatoid arthritis
- Autism
- Eosinophilic disorders
- ITP
- Chronic fatigue
- Depression


Green: beneficial effect FMT in RCT
Blue: beneficial effect FMT in case series
Black: association between gut microbiota and disease from experimental/observational studies

Smits LP, et al. Gastroenterology 2013
FMT Effective for Refractory Crohn’s Disease
Pilot study of mid-gut FMT

- 49 CD patients
- 86.7% (26/30) improved, 76.7% (23/30) in remission
FMT and Metabolic Syndrome

Obese

Lean

relative amount of two bacteria

Firmicute
Bacteroidetes
Transfer of Intestinal Microbiota From Lean Donors Increases Insulin Sensitivity in Individuals With Metabolic Syndrome

Vrieze A, et. al. Gastroenterology 2012
What does the future hold for FMT?
FMT Suitable for Mainstream Medicine

• Identifying a suitable donor

• Material processing
  • Take the smell out of it
  • Separate the microbial fraction from fecal matter
  • Freeze microbial fraction
  • Less aesthetically challenging
  • Bank material to allow testing (versus fresh)

• Development of a lyophilized preparation
  • Stored at room temperature
  • Encapsulated

• Reimbursement issues
A more appealing FMT?  
The RePOOPulate Trial

- Stool substitute preparation
- Purified intestinal bacterial cultures derived from a single healthy donor
- 33 species of commensal and beneficial bacteria
- 350 billion CFU in 100 mL of water into the colons of two people with rCDI
- Both patients
  - Reverted back to their normal bowel pattern (2-3 days)
  - Remained symptom-free for up to 6 months
SER-109, the “Ecobiotic”

- Ecobiotic
  - Combinations of a small number of selected discrete organisms
  - Work by enabling a shift from a disease state to one of health

- Single-arm, open-label clinical trial in rCDI
  - About 30 capsules, divided in 2 consecutive days
  - 29 of the 30 patients improved; no recurrences

- Results of a larger clinical trial awaited

- Development for rCDI, primary CDI, MDRO, IBD, Metabolic disease
Scientists want to mine our poop for gold

- 7 million tons of biosolids/year
  - 60% fertilizers
  - Rest incinerated or buried

- ASU study
  - Platinum, silver and gold
  - $13 million
  - Commercially mineable amounts of gold

Stone Motherboard 2015
Gut Microbiome and Us

• Rapid environmental transition and modern lifestyles are likely driving changes in the biodiversity of the human gut microbiota

• Clear effects on physiologic, immunologic and metabolic processes

• Aberrations in gut microbiome have capacity for multisystem effects

• Numerous disorders associated with dysbiosis

• Causality question

• Need a better understanding of the normal environment
Gut Microbiome and Us

• We are what we eat . . .
• We are what our gut microbiome is
• Using gut microbiota for management of health and illness needs further exploration

• Gut microbiome clinics of the future
  • Individual gut microflora assessment (matched with human microbiome)
  • Diagnostic, prophylactic and therapeutic target for various health issues
  • Infant evaluation
  • Therapeutic manipulation → better profiles to improve health
Gut Microbiome in Health and Disease

Gut microbial balance

Host physiology

Metabolism

Carcinogenesis

Bowel diseases

Obesity

Chronic disease

Therapeutic microbial manipulation

Healthy ‘organ’