Microbiota: Agents for Health and Disease
Dr. B. Brett Finlay

Talk outline

- A general overview:
  - Several aspects of microbiota
  - Various contribution in health and disease
  - Potential manipulation
  - Interface with the immune system and its development

What is the microbiome?

- All the microbes (microbiota) in and on our bodies
  - Includes bacteria, viruses, and eucaryotes
  - Microbiota used to be called "normal flora"
- Vast numbers on body sites exposed to environment
  - Not usually inside tissue
  - One gram feces contains > world’s population ($10^9$)
  - 10 trillion human cells, 100 trillion bacteria
  - >100x more genetic material in microbes than human genome!
  - Many thousand species (yet only about 100 are pathogens)
Where are they?

- On body surface, not inside (blood, deep tissue sterile)
- Mainly in gastrointestinal tract
- Flora different at different sites
  - Depends on environment

Microbiota numbers at various body locations

- Scalp = $1 \times 10^6$
- Mouth = $1 \times 10^9$
- Skin = $1 \times 10^2$
- Armpits and groin = $1 \times 10^7$
- Eyes = $1 \times 10^2$

Who are they?

- Two main phyla in intestine
  - Firmicutes and Bacteroidetes
- At phyla level composition is similar between humans and mice
- Much individual variation, many species
  - Thought to be core microbiome of 130 species, plus many others
### Microbiota: Agents for Health and Disease
Dr. B. Brett Finlay

<table>
<thead>
<tr>
<th>Eubacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroidetes (CFB) Gm(-)</td>
</tr>
<tr>
<td>Firmicutes Gm(+)</td>
</tr>
<tr>
<td>Proteobacteria Gm(-)</td>
</tr>
<tr>
<td>Actinobacteria</td>
</tr>
<tr>
<td>Cyanobacteria</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
</tr>
</tbody>
</table>

| Bacteroides Flavobacteria Chlorobi |
| Clostridium Bacillus Lactobacillus Streptococcus Enterococcus Etc. |
| E. coli Proteus Salmonella Etc. |

#### How to study the microbiota?

- **Viruses**
  - Not much known, use viral DNA chips
- **Protozoa**
  - Culture, DNA techniques, not well known
- **Bacteria**
  - Historically cultured bacteria
    - Only a tiny fraction can be grown
      - Strict anaerobes
      - Need community to grow?

#### Methods to study bacterial microbiota

- Besides selective plating, can stain DNA to get total numbers and idea of population
- Microbiota from feces of mouse stained with cybergreen DNA stain
DNA based methods to study bacterial microbiota

- Use fluorescently tagged DNA oligonucleotides to label 16s rRNA sequences (phyla level)
- Various techniques to PCR amplify 16s rRNA sequences using degenerate primers
  - Can cut these products with restriction enzymes and analyze lengths e.g. Terminal Restriction Fragment Length Polymorphism (TFLRP)
  - Sequence library of 16s rRNA clones
    - Powerful way to get list of species
- Metagenomics
  - Sequence all microbial DNA
    - Often don't know species it came from

Colonization is immediate and for life

- Acquired at birth
- From environment
  - Ingest food, fluids, inhalation
- Microbiota established rapidly
  - Stabilizes later

Normal flora composition affected by:

- Diet
  - Infant, vegetarian, etc.
- Infection
  - Diarrhea
- Antibiotics
  - More susceptible to secondary infections
Effects of different antibiotics on gut microbiota in mice

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>CFB  (Bacteroidetes)</th>
<th>Firmicutes+others</th>
<th>Proteobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Streptomycin 450mg/L</td>
<td>72 (13)</td>
<td>28 (13)</td>
<td>0%</td>
</tr>
<tr>
<td>Tetracycline 250mg/L</td>
<td>24.6 (15.9)</td>
<td>25.5 (15.9)</td>
<td>50%</td>
</tr>
<tr>
<td>Vancomycin 50mg/L</td>
<td>95.0 (3.9)</td>
<td>1.3 (1.6)</td>
<td>3.7 (3.6)</td>
</tr>
</tbody>
</table>

What do the microbiota do for us?

- **Microbial antagonism**
  - Plugs up sites, consumes nutrients, produces inhibitory substances, affects pH and oxygen
- **Nutritional benefits**
  - Vitamin K, B12
  - Steroid metabolism (breaks down bile acids)
  - Organic acid production
  - Food breakdown
- **Stimulate and enhance host defenses**
  - Need normal flora to develop normal immune system

What are the harmful effects of microbiota?

- **Pathogenic potential**
  - If introduced into other body sites
    - Urinary tract infections, flesh eating disease, septic shock, etc.
  - If host status changes (immunocompromised, nosocomial)
- **Gaseous byproducts**
  - Fermentation byproducts
  - Hydrogen disulfide, methane
  - 300 ml/day in gas produced
- **Other diseases**
  - Changes in lifestyle

The screen versions of these slides have full details of copyright and acknowledgements.
Hygiene hypothesis

- Do we live too cleanly in childhood (developed countries only)?
- Last 50 years of infectious diseases: all the major diseases have plummeted (rheumatic fever, hepatitis A, tuberculosis, mumps, measles)
- For other diseases mainly immune mediated there is a profound increase (Crohn’s disease, multiple sclerosis, type-1 diabetes, asthma)


Microbiota and diseases

- Obesity
  - Increased proportion of Firmicutes
  - Related to ability of microbiota to harness energy from food?
- Inflammatory Bowel Diseases
  - Microbial community imbalances
  - Increased Proteobacter, depleted Firmicutes and Bacteroidetes

Microbiota and diseases (2)

- Type I Diabetes
  - Interaction of intestinal microbes with innate immune system
- GI Cancers
  - H. pylori
  - Association of various species with colorectal cancer
- Oral diseases
  - Cavities and gingivitis disease
  - Most common infectious disease worldwide
Microbiota and diseases (3)

- Allergy-like (atopic) diseases
  - Eczema, allergies, asthma
  - Hygiene hypothesis
  - Induction of tolerance (early exposure)
  - Antibiotic treatment, C section increase rates of asthma
- Pseudomembranous colitis
  - Follows antibiotic treatment (which alters gut microbiota)
  - Caused by *Clostridium difficile*
  - Fecal transplants shown to improve outcome

Microbiota and the immune system

- Recently realized microbiota plays a key role in immune system development
  - Germ free (microbiota free) animals have poorly developed immune system
  - Activation of Toll-like receptors (TLRs) needed for development
- Segmented Filamentous Bacteria (SFB) are needed for Th17 cells
  - Critical T cell lineage
  - Germ free mice lack Th17 cells
  - Different mouse suppliers, antibiotics affect Th17 levels
- Very recently shown that Treg cells affected by microbiota

Ways to manipulate the microbiota

- Probiotics
  - Live bacteria such as *Lactobacilli* consumed orally
  - Some protective benefits
- Prebiotics
  - Sugars and other foodstuffs used to alter microbiota
- Immune modulators
  - Inflammation affects microbiota
- Antibiotics
  - Would increase resistance
Ways to manipulate the microbiota (2)

- Phage therapy
  - Target specific population (resistance rapidly)
- Fecal transplants
  - Used in *C. difficile* infections
  - May need to deplete current microbiota
- Use microbiota products
  - A bacterial polysaccharide from *Bacteroides fragilis*
    affects T cell population and Th1/Th2 balance
- Need other methods

Microbiota: agents for health and disease
The HST series

- The human microbiome
  - Human Microbiome Project,
    methods used to study, metabolomics
- Microbiota and disease
  - Many diseases discussed
- Microbiota and host responses
  - Immune development, learning, etc.
- Ways to manipulate the microbiota