Microbiome III Host interactions

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Bacteria outnumber our own cells 10 to 1

Who is in control?

Human microbiome project
Host-Microbiome genes proportion

BACTERIA

~ 8,000,000 genes
~ 99% beneficial
~ 1% pathogenic

FUNGI

~ 500,000 genes

ARCHAEA

~ 80,000 genes

HUMAN

~ 22,000 genes

VIRUSES

Foster JS et al. Life 2014, 4(2), 250-266
Human microbiota: distribution of bacteria, viruses, fungi in the host

Bacteria in the gut

3 pounds of bacteria
1000 species
The characteristics of human microbiota change over time in response to varying environmental conditions and life stages.

The intestinal microbiome is dominated by three phyla: the **Firmicutes** (Gram-positive), **Bacteroides** (Gram-negative) and **Actinobacteria** (Gram-positive).

### Role of the microbiome in the healthy gut

- Competition for nutrients with pathogens
- Competition for space with pathogens
- Production of bacteriocins
- Induction of antimicrobials and mucus production by intestinal cells
- Synthesis of nutrients, vitamins and metabolites
- 'Priming' of systemic immune effector cells
The human microbiome: at the interface of health and disease

Effect of maternal exposures
- Environment
- Antiseptics
- Antibiotics
- Diet
- Other hosts
- Epigenetics

Oral (pre-mastication of food)
Mammary, through breastfeeding (selection)
Cutaneous (contact with skin)
Vaginal (passage through birth canal)

Dental amalgam
Bottle feeding
Early/extensive bathing
Early-life antibiotics
Caesarean section

Nature Reviews | Genetics

Experimental pipeline for simultaneous analysis of the host transcriptome and bacterial microbiome and metatranscriptome

Donovan SM et al., Noninvasive molecular fingerprinting of host–microbiome interactions in neonates
Impact of the microbiota on the host
Gut Microbiota Imbalance

- Immune response to influenza virus infection
- Experimental immune encephalomyelitis
- Development of the immune system
- Inflammatory bowel disease
- Mucosal immunity
- Gut microbiota
- NAFLD
- Obesity
  - Metabolic syndrome
  - Insulin resistance
- Autoimmune arthritis
- Hepatobiliary-pancreatic autoimmune disease
Diseases influenced by gut microbial metabolism

Kinross JM. Genome Medicine 2011
The human microbiome: at the interface of health and disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>Relevant finding</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psoriasis</td>
<td>Increased ratio of Firmicutes to Actinobacteria</td>
<td>88</td>
</tr>
<tr>
<td>Reflux oesophagitis</td>
<td>Oesophageal microbiota dominated by gram-negative anaerobes; gastric microbiota with low or absent <em>Helicobacter pylori</em></td>
<td>75,133</td>
</tr>
<tr>
<td>Obesity</td>
<td>Reduced ratio of Bacteroidetes to Firmicutes</td>
<td>17,31</td>
</tr>
<tr>
<td>Childhood-onset asthma</td>
<td>Absent gastric <em>H. pylori</em> (especially the cytotoxin-associated gene A (cagA) genotype)</td>
<td>96,134</td>
</tr>
<tr>
<td>Inflammatory bowel disease (colitis)</td>
<td>Larger populations of Enterobacteriaceae</td>
<td>113</td>
</tr>
<tr>
<td>Functional bowel diseases</td>
<td>Larger populations of <em>Veillonella</em> and <em>Lactobacillus</em></td>
<td>135</td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>Larger populations of <em>Fusobacterium spp.</em></td>
<td>101,102</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>Gut-microbiota-dependent metabolism of phosphatidylcholine</td>
<td>136</td>
</tr>
</tbody>
</table>
Association of microbiota with diseases outside of the gastrointestinal tract

Inna Sekirov et al. Physiol Rev 2010;90:859-904

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Human Microbiota in Health and Disease
Impact from Intestinal Interactions

de Vos WM et. al. Self Care 2012;3(S1):1-68
Schematic representation of diet, microbes, and host interaction at gut level

The chemical dialogue via low molecular weight metabolites, peptides, and proteins between cell-cell and host-microbes leads to the metabolite production in different body fluids which could be considered as disease biomarkers.
The core Human microbiome
While some intestinal bacteria can process a primary food source, other bacteria are dependent on metabolic products (secondary food source) produced by these bacteria.

Other members of the microbiome play a role in the removal of waste products.
While some intestinal bacteria can process a primary food source, other bacteria are dependent on metabolic products (secondary food source) produced by these bacteria. Other members of the microbiome play a role in the removal of waste products.

Components of the microbiome express conserved molecular structures termed microorganism-associated molecular patterns (MAMPs) which are sensed by pattern recognition receptors (PRRs) – such as Toll-like receptors (TLR) – expressed on IECs, which continuously respond to these compounds in order to orchestrate the immune response.

MAMP and metabolite release are a signal for intestinal cells to continuously produce and secrete mucus, antimicrobials and cytokines. Nutrients are also able to activate the immune system directly. M cells are the most important cells in sampling antigens and bacteria from the gut lumen by transferring the material to the underlying Peyer’s patches. Dendritic cells (DCs) process and present antigens of T cells and B-cells in the Peyer’s patch or migrate to mesenteric lymph nodes to do this. B-cells that mature become IgA-secreting plasma cells.

This dimeric soluble IgA (slgA) is transported through epithelial cells into the mucosal surface. slgA is not only important in eradication of pathogens but also in transporting antigens back into the gut lumen in order to maintain homeostasis. Besides M cells, intraepithelial DCs are also involved in direct uptake of antigens and bacteria from the mucosal surface.
Host microbiome interactions

The impact of the intestinal microbiome does not stop at the gut.

Metabolites, such as short-chain fatty acids (SCFA), are partly taken up by IECs while another portion enters the systemic circulation.

SCFAs and butyrate have an anti-inflammatory effect on leukocytes.

Additionally, components of the intestinal microbiome are translocated into the systemic circulation and continuously prime neutrophils leading to enhanced capability of these cells to kill pathogens.
Pro-inflammatory mediators increase the mucosal permeability by altering the integrity of tight junctions between enterocytes. Increased apoptosis of intestinal epithelial cells is correlated with sepsis-induced mortality. Loss of the intestinal epithelial barrier function potentially results in translocation of the intestinal microbiome into the circulation.

Use of antibiotics can kill members of the intestinal microbiome either directly or indirectly. Indirectly, if these bacteria are dependent on a food source derived from bacteria that are directly affected by antibiotics. Antibiotic treatment decreases the number of intestinal bacteria resulting in diminished release of MAMPs and other metabolites. As a result, fewer antimicrobial molecules, such as defensins and lectins, are produced by Paneth cells, less mucus is produced by goblet cells etc., which makes the host more susceptible to invasion by intestinal pathogens.
Factors leading to microbiota dysbiosis and disease

Diet

GI physiology
- Transit
- Secretion/absorption

Genetic background

Immune system

Antibiotics

Microbiome

Pathogens
The role of microbiota in inflammatory bowel disease (IBD) pathogenesis

Host Genetics (e.g., NOD2, IBD5, IL23R, ATG16L1)

Defects in:
- Innate immunity
  - ↓AMP
  - Defects in mucosal barrier
  - Abnormal mucins expression
- Autophagy
  - Mutations in autophagy loci
  - Implications for granuloma formation
- Phagocytosis
  - Altered phagosome function

Proinflammatory response → Mucosal damage → (Further) Dysbiosis

Microbiota
↑Enterobacteriaceae
  - E. coli (especially AIEC)
  - F. prausnitzii
  - Colitogenic microbiota
  - Dysbiosis

Defective microbiota control → Dysbiosis

Microscopically
- Loss of barrier integrity
- Induction of pro-inflammatory cytokines
- Lesions in intestinal epithelium
  - Fibrotic scarring

Symptomatically
- Diarrhea/weight loss
- Abdominal pain & cramping
- Profuse GI bleeding
  - Obstructions

Inflammation

Ulceration/Fibrosis

Metaplasia/Cancer

Inna Sekirov et al. Physiol Rev 2010;90:859-904

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The evolution of antibiotic resistance through natural selection

1. A bunch of bacteria, including a resistant variety...

2. ...get bathed in antibiotics. Most of the normal bacteria die.

3. The resistant bacteria multiply and become more common.

4. Eventually, the entire infection evolves into a resistant strain.

normal bacterium  dead bacterium  resistant bacterium
When a clinical condition is clearly caused by disturbance of the normal gut microbiota, such as in *C. difficile* Infections (CDI), one possibility is to restore the normal intestinal microbiota through ‘transplantation’ of normal donor colonic or duodenal infusions.

This ‘faecal transplantation’ has been tried in hundreds of cases over the last 50 years and seems to be characterized by a high degree of success.

Recent studies have allowed the application of modern analysis to characterise the changes of microbiota on transplantation.

Deep and high throughput analysis of the microbiota for several months after transplantation confirmed the correction of the microbiota:

- Initial low diversity in patients with CDI
- Stability of the newly established diverse community
- A clear shift in the microbiota of the recipients towards the donor signature, correcting the effect of the patients’ gram-negative pathogens
*Clostridium difficile*

Gram +, Firmicutes, obligate anaerobe, rod-shaped
Fecal microbiota transplant for patients with recalcitrant Clostridium difficile infections

Obesity an international disease

Clinic Compare UK 2015
Obesity and antibiotics

Obesity trends in US Adults, 2010
Source: CDC Behavioral Risk Factor Surveillance System.

Antibiotic prescriptions per 1000 persons, 2010
Source: L Hicks, TH Taylor, RJ Hunkler. NEJM 2013, 368:1461.
Germ free animals

Mouse Isolation Chamber

Free of bacteria, viruses, fungi, and parasites

Mono association with a certain bacteria/pathogen or transfer a group of bacteria or stool sample from human or mice to demonstrate causality of the disease
Schematic representation of areas of the rodent system that are significantly impacted by the absence of a normal microbiome

Intestinal
- Increased muscular tissues, altered myenteric nerves, increased transit time of contents, increased enteroglucagon cells, altered enzyme expression, no urobilin in urine and increased bilirubin in faeces.

Exocrine
- Higher trypsin and chymotrypsin
- Higher mucoproteins and mucopolysaccharides.

Vascular
- Lower iodine uptake by thyroid. Hyporesponsive to epinephrine, norepinephrine and vasopressin.

Endocrine
- More susceptible to a wide range of pathogens e.g. Shigella flexneri, Listeria spp., Leishmania spp., Coxsackie B

Infection
- Decreased IgA, increased 5-HT in small intestine, decreased MHC-II on small intestinal cells, less NO in small intestine, less histamine in the intestine, MLN are smaller and do not have germinal centres, low Ig levels, altered adaptive immune response.

Immunity

Metabolism
- Increased urea and no ammonia in intestines, increased excretion of amino acids in urine, more nitrogen in faeces and caecal matter, excrete low amounts of acetate and caecal contents have more hexosamines.

Morphology
- Intestinal mass and surface area decreased, villi of small intestine are decreased, crypts of lamina propria are shorter, lamina propria is thinner, cellular renewal is lower, cecum is larger and caecal wall is thinner.

Epithelia
- Lower rate of cell turnover in the small intestine and Peyer’s patches, increased number of goblet cells in cecum and altered lectin composition in colonic mucus.
Germ free mice adopt the phenotype of microbiota donor
Germ free mice adopt the phenotype of microbiota donor

![Diagram showing the effects of normal microbiota development versus disrupted microbiota on germ-free mice.](image)

- Control: Normal microbiota development
- Disrupted microbiota: Loss of early-life protective bacteria
- Low-dose penicillin
- Microbiota transfer to germ-free mice
Fecal transplantation studies in mice show that transferring the microbiota from lean and fat mice to germ-free mice induces greater weight gain in those receiving the ‘fat’ microbiota.

The discovery of this obesity-associated microbiome, raises the possibility of using transplanted microbiota to influence metabolic processes in humans.

In a controlled study (autologous transplantation) examining the effect of fecal transplantation from lean subjects to those with metabolic syndrome, results showed significant improvement of whole body insulin sensitivity at 6 weeks.

Smits LP et al. Gastroenterology 2013
Germ free mice adopt the phenotype of microbiota donor
Transkingdom control of microbiota diurnal oscillations control metabolic homeostasis

- Intact host circadian clock and feeding habits
- Detoxification, motility, environmental sensing
- Energy metabolism
- Cell growth, DNA repair

- Loss of diurnal rhythmicity
- Impaired host circadian clock and feeding habits (jet lag / shift work)
- Dysbiosis
- Obesity, glucose intolerance

Metabolic homeostasis

Elinav E. and colleagues  Cell. 2014 Oct 23;159(3):514-29
Transkingdom control of microbiota diurnal oscillations control metabolic homeostasis

Subject 1 (10 hours shift)
Subject 2 (8 hours shift)

Relative abundance (%)

Before jet lag | During jet lag | After jet lag
---|---|---
Subject 1 | Subject 2

Bacteroidetes | Firmicutes | Other

Subject 1 (10 hours shift)
Subject 2 (8 hours shift)

Relative abundance (%)

Before jet lag | During jet lag | After jet lag
---|---|---
Subject 1 | Subject 2

Firmicutes | Bacteroidetes | Other

Elinav E. and colleagues Cell. 2014 Oct 23;159(3):514-29
How to restore a healthy microbiota

Probiotics: beneficial live bacteria and yeast

Prebiotics: non-digestible fibers beneficial for “good“ bacteria
Probiotics as protective – Mechanisms


Modulation of Tight Junctions

Damage to epithelial tight junctions by inflammatory processes (A) or pathogens (B) can lead to infection and sepsis, as well as impaired nutrient uptake. The ability of lactobacilli to up-regulate tight junction proteins helps prevent these adverse events (C).
Probiotics as protective – Mechanisms

<table>
<thead>
<tr>
<th>Mechanism</th>
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<tbody>
<tr>
<td><strong>Biosurfactant Production</strong></td>
<td>Biosurfactants are produced by lactobacilli and their presence on the mucosal surface helps prevent adhesion and infection by the pathogenic organisms.</td>
</tr>
<tr>
<td><strong>Bacteriocin and Hydrogen Peroxide Production</strong></td>
<td>Lactobacilli produce substances that can inhibit the growth or kill pathogens. Illustrated here are hydrogen peroxide and bacteriocins.</td>
</tr>
</tbody>
</table>
Bacterial communication

Signaling effects
Bacteria communicate through a number of signaling mechanisms including quorum sensing. In this illustration, the Lactobacillus signaling molecules down-regulate toxin production in the Gram negative pathogen (for example *E. coli 0157:H7* in the gut) and Gram positive pathogen (for example *S. aureus* on the vaginal surface).

Pathogen-host-microbiota interactions and outcome of infection

Inna Sekirov et al. Physiol Rev 2010;90:859-904

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The human microbiome: at the interface of health and disease

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Microbiota-dependent carcinogenesis

Gastric cancer

Colon cancer

Inna Sekirov et al. Physiol Rev 2010;90:859-904

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Signalling between the intestinal microbiota, the host, and incoming pathogens

Inna Sekirov et al. Physiol Rev 2010;90:859-904

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Eosinophilic Esophagitis across the world
Eosinophilic Esophagitis (EoE)

- Early life exposures associated with increased odds of developing pediatric-onset EoE
  - Antibiotic use in infancy (6 times odds)
  - Cesarean delivery
  - Preterm birth
  - Formula-only or mixed feeding

Jensen et al. JPGN 2013
Rationale

Patients with allergic diseases (AD) have an alteration of their skin microbiome

Patients with asthma have an alteration of their lung microbiome

Kong HH et al. Genome Research 2012
Goleva E et al. Am J Respir Crit Care Med. 2013
Haemophilus is significantly increased in untreated EoE
Th2 cytokines increase the expression of *Haemophilus* receptors

*Fillon SA et al. unpublished data*
IL-5 and LPS increases the expression of CEACAM-1 receptor

Co

LPS+IL-5

CEACAM-1

Actin

CEACAM-1/Actin

Fillon SA et al. unpublished data
Questions?

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