Medicinal Chemistry of The Human Microbiome

Lecture 7
Outline

• What is gut microbiome and its major functions
• Classes of bacteria living in our organism
  • Oral cavity
  • Skin
  • Urinary tract
  • Gut
• Diseases associated with microbiome: Inflammatory bowel disease
• Medicinal tools to “heal” gut microbiome
• Brain-gut connection
• Tools to study gut microbiome and its function
Microbes are all over us

There are millions of microbes per square inch on your body.

Thousands of different species on the skin alone.
Some thrive on dry patches of the elbow, others thrive in moist environment of armpit.

It is estimated that there are more microbes in your intestine than there are human cells in your body!
What is the Human Microbiome?

Microbe: tiny living organism, such as bacterium, fungus, protozoan, or virus

Microbiome: collectively all the microbes in the human body; a community of microbes

Biofilm: a community of microbes that live together on a surface

https://www.youtube.com/watch?feature=player_detailpage&v=5DTEnvDWyvM
Microbes in the Human Microbiome include species from each major domain.
What features distinguish the microbial domains?

**Bacteria**
- Have no nucleus or membrane bound organelles
- Often sphere (cocci) or rod (bacillus) shape, but others as well

**Archeabacteria**
- Have no nucleus or membrane bound organelles
- Can look similar to bacteria or drastically different shapes, such as flat and square
- Have some metabolic similarities to eukaryotes

**Eukaryotes**
- Have a true nucleus and membrane bound organelles
- Wide variety of shapes. They have a cell wall and form spores during reproduction

https://www.youtube.com/watch?feature=player_detailpage&v=TjPvSpKh5oI
Microbes are normally found in and on the human body. The following sites are “hotspots” for microbial life:

- Nasal
- Oral
- Skin
- Gastro-intestinal
- Urogenital

Some microbes are **native**, normally found in the body.

Some microbes are **introduced**, suddenly arriving at a new residence in the body.

Let’s explore these five regions.
What’s Happening in the Nose?

The nose is a primary defender against inhaled pathogens

Mucous lining trap inhaled microbes

Inflammation from viral infection and allergic reactions

Inhaled medicines and oral antibiotics

There is a delicate balance of microbes that are maintained to keep that environment healthy. Weakened immune systems can throw off that balance and allow the wrong microbes to grow out of control.
The interior lining of the nose contains mucous secreting glands. A wide variety of microbes are normally found there. Here’s a few:

- *Staphylococcus epidermidis* bacteria forms a biofilm that coats the mucosal lining.

- *Staphylococcus aureus* bacteria is fine when kept under control by a protease found in *S. epidermidis*, but if left to grow out of control, *S. aureus* can become pathogenic and cause infection.
Nose

- **Aspergillus** fungal spores are often inhaled through the nose. If the immune system fails to clear these, mold can grow in the lungs.

- *Corynebacterium accolens* bacteria is rarely a pathogen, but if it enters the bloodstream due to a torn blood vessel, it can cause serious infections.
What’s Happening in the Oral Cavity?

A wide variety of microbes regularly enter the oral cavity. Saliva, pH, temperature, and the immune system prevent many species from surviving. Oral antibiotics inhibit growth.

Brushing and flossing teeth clears some built up biofilm. Symbiosis of the oral microbes that are able to survive these conditions form an elaborate scaffold that lives on the tooth enamel and at the interface with the gums. It forms a barrier for incoming bacteria.
Oral Cavity

The oral cavity has a wide variety of microbes normally found there. Here’s a few:

*Fusobacterium sp.* bacteria is a larger bacteria that helps form a scaffold for many other bacteria in the oral biofilm.

*Streptococcus mitis* bacteria typically forms a biofilm on the hard enamel surfaces of the teeth. If gums get inflamed, it can enter the bloodstream and cause infection.
Oral Cavity

- *Prevotella sp.* bacteria have natural antibiotic resistance genes. They can attach to epithelial cells or other bacteria and cause larger infections in inflamed areas.

- *Candida albicans* fungus can cause oral infection known as thrush
What’s Happening on the Skin?

There are several skin environments: oily, dry, moist. Some microbes prefer one over another.

Microbes hide in crevices to recolonize skin after washing with soap.

The skin has natural defenses including slightly acidic sweat and antimicrobial peptides.

Antibiotic washes and oral antibiotics disturb normal balance of microbes on the skin.

There is a normal balance of microbes on the skin that protect introduced microbes from harming us. Damaged skin gives opportunities for microbes to invade the bloodstream and cause serious illness.
• *Propionibacterium acnes* bacteria colonizes healthy pores, but if pores become clogged, it grows out of control.

• *Staphylococcus epidermidis* bacteria normally colonizes on the skin. But when *P. acnes* clogs pores, *S. epidermidis* also grows out of control in the infected pores.

• *Staphylococcus aureus* bacteria can also infect clogged pores like *Staph epidermidis*. Even worse, many antibiotic resistant strains of *Staph aureus* make it difficult to treat the infection.
Skin

*Trichophyton* and *Microsporum* fungi feast on keratin in the skin and cause ringworm fungal infections.
Burns and Burn-related infection

• ‘Burns’
  – Heat and chemical degradation

Why burns could be so dangerous?

https://www.youtube.com/watch?feature=player_detailpage&v=Dsvtzwp4nG8

https://www.youtube.com/watch?feature=player_detailpage&v=ZlbanGBgecc
What’s Happening in the Urogenital Tract?

Urinary system almost sterile due to urea and other chemicals

Urine often flushes out microbes that find their way in

Introducing a catheter into the urethra can introduce microbes directly into the bladder, where a biofilm can grow and cause bladder infection.
The urinary tract is normally sterile due to urine flushing out the tract.

But, *Escherichia coli* from GI tract can infect urinary tract due to poor hygiene and contamination from nearby GI tract opening.
The Gut Microbiota

- Complex community of microbes – estimated to contain 200 trillion cells
- > 1000 diverse microbial species
- 10 x the number of human cells in our body
- Gut microbiome is 150 x larger than the human genome

https://youtu.be/EEZSuwkx7Ik
Functions of Gut Bacteria

• It helps the body to digest certain foods
• It helps with the production of some vitamins
• It helps us combat aggressions from other microorganisms
• It plays an important role in the immune system
Gut Microbiota - Functions

- Prevents colonization by pathogens
- "Educates the immune system"
- Metabolic role
  - Caloric salvage
  - Produces
    - SCFA
    - Vit K and folate
- Participates in drug metabolism
  - Activates 5-ASA (a drug anti-inflammatory drug used to treat inflammatory bowel disease, including ulcerative colitis, or inflamed anus or rectum, and to maintain remission in Crohn's disease).
Gut Microbiota

• Colonization of the gastrointestinal tract begins immediately after birth

• Initial bacterial colonization (normal) starts from a “Germ free” intrauterine environment and is populated through maternal vaginal/fecal flora and oral feeding (breast milk vs formula)

• Complete adult colonization: by 3 yrs of life

• For a given individual, the fecal microbiota remains remarkably stable over a person’s lifetime
Is My Gut Microbiome the Same as Yours?

The number and amount of the many different microbes can vary greatly from person to person.
Factors affecting Gut Microbiome

Key characteristics of the microbiome, including stability, resiliency and complexity, are influenced over time from infancy to adulthood and old age.
How Does the Brain and Gut Connect?
What is The Brain in the Gut?

• The Enteric Nervous System is embedded in the lining of the GI system:
  – Esophagus
  – Stomach
  – Small intestine
  – colon
Neurotransmitters: Serotonin

- Although manufactured in the brain, 90-95% of our supply is found in the digestive tract
- Vital for communication
- Important to motility and sensitivity to gut sensations
- It is also a “feel good” hormone
20% of vagus nerve fibers send instructions from the brain to the stomach

These signals control:
- Gastric acid secretion
- Digestive enzyme secretion
- Gastric capacity
- Blood glucose

80% of vagus nerve fibers send instructions from the stomach to the brain

These signals control:
- Satiety (Hunger)
- Satiation (Fullness)
- Energy Metabolism
Gastrointestinal Function

• Appears particularly influenced by stress.
• Common symptoms due to stress are heartburn, indigestion, nausea and vomiting, diarrhea, constipation and abdominal pain.
How Stress Impacts Your Gut

- Decreased nutrient absorption
- Decreased oxygenation to your gut
- Four times less blood flow to your digestive system
- Decreased metabolism
- Decreased enzymatic output
- Alterations in gastrointestinal motility
- Negative effects on regenerative capacity of gut
Recent animal studies suggest that different types of psychological stress can affect the composition of gut bacteria (this community called microbiome) and this communication
Microbiome

Alterations in gut microbiota have been linked with:

- FBD (functional bowel disorder)
- IBD (Inflammatory bowel disease)
- CDI (Clostridium difficile infection)
- Celiac disease
- Allergies
- Autism

Metabolic diseases
- Obesity
- Cancer
- Type I DM
- NASH
- Depression
Dietary/microbial interactions impact intestinal, hepatic and vascular inflammation

J Goldsmith and RB Sartor
J. Gastroenterology 2014
Inflammatory bowel disease (IBD)

• chronic inflammation of the gastrointestinal tract

• two main forms:
  1. Crohn`s disease
     – affects all layers of the bowel wall
     – granuloma formation in up to 60% of patients
  2. Ulcerative colitis
     – affects superficial mucosal layers

• no pathogen has been conclusively shown to be the causative agent

• incidence is the highest in developed countries
Gut Microbiota – IBD (Inflammatory bowel disease)

- Effects ~1.3% of population
- 30–50 percent reduced biodiversity of commensal bacteria

Pathogenesis – linked to inappropriate activation of GI immune system toward the gut microbiota in genetically susceptible hosts & under the influence of environmental factors

Copyright: https://www.google.com/search?q=Inflammatory+bowel+disease&source
Gut Microbiota – IBD (*Inflammatory bowel disease*)
Involvement of the microbiota in regulating the balance between $T_H$ and $T_{Reg}$ cell subsets in the gut.

Intestinal bacteria direct the differentiation of both pro- and anti-inflammatory T cell populations and may therefore play a crucial role in IBD.
Summary

• the microbiota promote the appropriate development of the immune system

• immune-mediated disorders seem to involve reduced $T_{\text{Reg}}$ cell activity

• absence of beneficial microorganisms (owing to dysbiosis) can lead to the induction of inflammatory responses and immune-mediated diseases

→ the microbiota plays an important role in supporting health and there are several way to control it
How can we change Gut Microbiota?

- Diet
- Antibiotics
- Prebiotics
- Probiotics
- Symbiotics
- FMT
Different bacterial species ameliorate the symptoms of IBD

Probiotics

- dietary microorganisms that are beneficial to the health of the host
- act on several cell types (epithelial cells, DCs, T cells)
- ability to limit inflammation by induction of $T_{\text{Reg}}$ cells
C. difficile Infection (CDI)

• Spore-forming, anaerobic, gram-positive
• Leading cause of healthcare-associated infectious diarrhea in US
  – 3 million cases per year in US along
  – Rates of CDI doubled
• Very high fatality rate
  – Majority > 65 y/o
• ~ 3.2 billion dollars excess cost of care in US along

Gastroenterology 2012 Nov;143(5):1179-1187
Novel solution to cure functional bowel disorder?

You guess?

Yellow Soup or Cappuccino?

https://www.youtube.com/watch?feature=player_detailpage&v=a_RlHIR4WUc
Fecal Microbiota Transplantation (FMT)

- **Definition**: Instillation of stool from a healthy person into a sick person to treat a certain disease

- **Rationale**: A perturbed imbalance in our intestinal microbiota (dysbiosis) is associated with or causes disease and can be corrected with re-introduction of donor feces
Early History of FMT

• 4th Century (Ge Hong):
  – Oral human fecal suspension (“yellow soup”) for severe diarrheal illnesses, food poisoning
• 16th Century (Li Shinzen): fermented fecal solution, dry feces - treated fever, severe diarrhea, vomiting and constipation
• 17th Century: Veterinary medicine
  – Fecal transfer for horses with diarrhea
• 1958: FMT enema
  – Eismann, et al. 4 patients with pseudomembranous colitis
  – “Dramatic” response within 48 hours
Human Microbiome and Obesity

The most effective treatment for obesity has to do with human microbiome!

Study: The right bacteria may help fight obesity

(Photo: Elizabethe Holland, Washington University School of Medicine, via AP)
What is that treatment?

Study: The right bacteria may help fight obesity

https://www.youtube.com/watch?v=t6y3yz0BiDE&feature=player_detailpage
Conditions Potentially Treatable By FMT

- Multiple sclerosis
- Chronic fatigue syndrome
- Non-alcoholic fatty liver disease
- Atherosclerosis
- Idiopathic thrombocytic purpura
- Insulin resistance/type 2 diabetes mellitus
- Obesity
- C difficile infection
- Irritable bowel syndrome
- Inflammatory bowel disease

Green: beneficial effect FMT in RCT
Blue: beneficial effect FMT in case series
Black: association between gut microbiota and disease from experimental/observational studies
How can we change Gut Microbiota?

- Diet
- Antibiotics
- Prebiotics
- Probiotics
- Symbiotics
- FMT
Probiotics

First described by Metchnikoff in 1907
Probiotics and Prebiotics

Probiotics
Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host

Prebiotics
Nondigestible substances that provide a beneficial physiological effect for the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria
Probiotics

Synbiotics

Products that contain both probiotics and prebiotics
Probiotics

• Global sales of Probiotics – 21.6 billion dollars in 2010, expected to reach 31.1 billion in 2015

• Fastest growing segment of the global dietary supplement & functional food industries

• Why?

• People are looking for ‘natural’ or non-drug ways to maintain health or treat disease
Clinical Trial: Prebiotics on Mood

Those taking the prebiotic had a reduced tendency to pay attention to negative information, which is a key component of anxiety and depression.

– They also had lower levels of stress hormone cortisol, which has also been connected to anxiety and depression.

Study: Probiotics for IBS

• Irritable bowel syndrome (IBS) is a common disorder of the intestines that leads to crampy pain, gassiness, bloating and changes in bowel habits.

• Adequate relief reported in 47% (11% in placebo)

• Improved global symptom score, pain, distension/bloating and stool urgency.

Guglielmetti S, et al. RCT: Bifidobacterium bidfidum MIMBb75 significantly alleviates IBS and improves QOL-a DBPCS. *Aliment Pharmacolo Ther.* 2011. 33(10)
Characteristics of Effective Probiotics

- Able to survive the passage through the digestive system.
- Able to attach to the intestinal epithelia and colonize.
- Able to maintain good viability.
- Able to utilize the nutrients and substrates in a normal diet.
- Non pathogenic and non toxic.
- Capable of exerting a beneficial effect on the host.
- Stability of desired characteristics during processing, storage and transportation.
A novel approach for imaging BSH activity *in vitro* and *in vivo*
Microbial Bile Salt Hydrolase (BSH)

- Required for the conversion of primary to secondary bile acids (BA)
- The ability of probiotic strains to tolerate BA is one of the important criteria for strain selection
- BSH activity of probiotics had been widely shown to mediate multiple beneficial effects to the host including lowering of plasma cholesterol levels and gallstones formation.
- However, no tools currently exist for real-time non-invasive imaging and quantification of BSH activity in live animals
Probiotic strain selection

• Has been generally based on \textit{in vitro} tolerance of physiologically relevant stresses: e.g., low pH, elevated osmolarity, and bile.

• However, none of these \textit{ex vitro} tests reflect the true complexity of the gut microbiota \textit{in vivo} making selection of beneficial probiotic strains very complex and inefficient.

• Current methods suffers from low sensitivity, necessity of invasive sampling and are not suitable for non-invasive real-time \textit{in vivo} analysis.

• Therefore, development of novel methodologies for the quantification of BSH activity both \textit{in vitro} and \textit{in vivo} are of significant interest for selection of novel BSH positive probiotic strains.
Bioluminescent imaging (BLI)

- Sensitive imaging modality

\[
\text{Firefly Luciferin} \xrightarrow{\text{Firefly luciferase}} \text{hv} \xrightarrow{\text{ATP, O}_2, \text{Mg}^{2+}} \text{Oxyluciferin} + \text{AMP, PP}_i, \text{CO}_2
\]

Chemical & Engineering News, 2006, 84, 36-38; Science, 2005, 309, 263
Schematic of the Bile Acid Caged Luciferin Assay (BALC)

Bile Acids:
Chenodeoxycholic, deoxycholic, lithocholic and cholic bile acids.
Cleavage of bile acid caged luciferin in faecal water

**In vitro enzymatic assay**

**Source of BSH:**
Faecal water from conventional (Conv) and germ free (GF) mice.

**Source of Luciferase:**
4T1-Luc cells

![Diagram showing the enzymatic assay process]
Does antibiotic treatment reduce BL signal from BACL’s?

Ampicillin, Vancomycin, Neomycin, Metronidazole; AVNM taken from Ayres et al., 2012.
Colonization of AVNM treated mice with BSH expressing bacteria

Novel tools for quantification of BSH activity may facilitate the development of BSH-active probiotics

First example of real-time non invasive imaging of BSH probiotic activity in life mice

*L. Plantarum* $10^8$ CFU/mouse
Probiotics in the Treatment of Gastrointestinal Disorders

- Diarrhea
- Acute infectious
- Antibiotic-associated
- *C. difficile*

- Lactose Intolerance

- FBD

- IBD
- Ulcerative colitis
- Crohn’s disease
- Pouchitis

*H. pylori* Eradication
Interplay Between Medicine and Microbes

Antibiotics

Kills infectious bacteria but also disrupts natural flora. Can result in yeast infections, digestive problems, etc.

Chemotherapy drugs

Gut flora has been shown to modify some drugs during metabolism. This causes many side effects, including upset stomach.
Use of Antimicrobial Products

How many do we really need?

But do we need some natural exposure to germs to keep our normal flora around?

Products kill germs to reduce infection

Will this allow “superbugs” that can barely survive these treatments to grow and become more prevalent...causing problems for the future?
C-sections and antibiotics have the largest impact

- Chronic illnesses like allergies, asthma, Celiac disease and type 1 diabetes are thought to be a result of lost microbes.

- Some scientists theorize that children born via C-section may miss out on important microbes that shape their immune systems and in turn their lifelong health.

- Antibiotics, rigorous hygiene practices and highly processed foods are also believed to have decreased the diversity of microbiomes in the developed world,

- "Every time we take an antibiotic, and especially during a very early age, the assembly of these communities is disrupted by the antibiotics. It's a tremendous effect because, by definition, antibiotics kill bacteria"

Read more at: “From ancient tribes to modern civilization, what do our microbiomes say about us? By Michelle Cohan, CNN” (taken from this article)
What are the current methods to study gut microbiome?

• Problem
  
  – 95% of gut microbiome bacteria can not be cultured!
What are the current methods to study gut microbiome?

• Proteomics
  – is the large-scale study of proteins, particularly their structures and functions.

• Metabolomics
  – small-molecule metabolite profiles

• Functional Imaging (new)
3 foods to keep you gut microbiome healthy!

https://www.youtube.com/watch?feature=player_detailpage&v=G04AZheLw6Q

https://www.youtube.com/watch?feature=player_detailpage&v=l7N3eB_7Exw
“All disease begins in the gut”

“Health is determined by the microbiota in our gut”

Hippocrates 460 BC – 370BC
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