The Human Microbiome: Friends and Foes

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Human Microbiome: What is it?

• Microbiome includes all the microscopic cells and their entire genetic capability that live in a specific environment.
• Human Microbiome refers to the microbiome of the surfaces in and on the human species.
• The species of the human microbiome living in one part of the body differs from species found in other parts of the body.
• Weight of human microbiome: 2-3 lbs.
Microbiome Research Nationally

- 2000-1 – Human Genome Project Completed
- 2008 – Human Microbiome Project launched
- 2015 – National Microbiome Initiative announced by the White House
- Privately, the American Gut Project was launched to involve citizens around the world in the analysis of their gut microbiome.
The Invisible Universe of the Human Microbiome

• https://www.youtube.com/watch?v=5DTrENDWvVM&feature=player_detailpage
Microbiota Genes are Important to Humans

Microbial genetic capabilities exceed that of the human host:

- Human genes in egg and sperm -- about 23,000 genes in total
- Microbiome genes in one human -- about 3 million genes or more in total
- Genes of our microbiota produce many enzymes that humans utilize.
- Some researchers now call the human plus its microbiome the **superorganism**.
Are we Human or More than Human?

• The average human body is estimated to contain approximately 10 trillion human cells total.
• The number of non-human cells in the human microbiome is estimated to be 100 trillion or more.
• If we do the math: A human is outnumbered 10 to 1 by microscopic species called the human microbiota.
• The human microbiota are the sum total of microscopic species that inhabit “you”.
• On earth, microbiota is estimated in millions of different species
• In a human, only about 1,000 different microbial species
Focus is on symbiotic relationship of human microbiome that varies:

• From one part of the body to another.
• From birth to adolescence to adult to senior
• From person to person
• From one family to another
• From one cultural group or nation to neighboring groups or nations
Terms for Microscopic Life

- Microorganisms are too small to be seen by the naked eye; their cells are visible by using various microscopes.
- microbiota
- microorganisms
- microscopic species
- microbe
- mycobiome (meaning fungi: yeast, molds, etc.)
Microbiota: What is it?

• Microbiota refers to all the species of microscopic organisms in an environment, including:
  – Bacteria: Archaeobacteria and Eubacteria
  – Fungi: Yeasts, Molds, Mushrooms, Others
  – Protozoa: Amoebas, Ciliates, Flagellates, Sporozoans
  – Viruses
The Three Domains of Living Organisms

• Archaea – most ancient species, found in extremely harsh environments.
• Bacteria – Include many large groups known as phyla, the ones that colonize humans belong in this domain.
• Eukarya – Organisms that have a true nucleus meaning the chromosomes are organized inside a membrane structure. Includes animals, plants, protozoa, algae.
• Humans live with members from each domain.
Comparative sizes of different kinds of microorganisms. Å (Angstrom). One Angstrom equals 1/10,000 (10^-4) of a micron (µ), or 1/10 millimicrons (0.1 nm). Ultraviolet (U.V.) extends the useful range of the light microscope only slightly, the electron microscope makes possible clear images of objects no more than 10 Å (1 nm) in size.
Bacterial Shapes and Arrangements

coccus, pl. cocci
rod, bacillus, pl. bacilli
spiral, helical, vibrio

 Diplo-
staphylo- (irregular cluster)
diplo-
strepto-
cluster (regular)
chain, strepto-
spore

 Singles

Chain
What do the Microbiota do?

- Produce digestive enzymes that humans don’t
- Microbially produced vitamins are produced and absorbed into human bloodstream
- Assist in maturing the human immune system
- Produce short-chain fatty acids (SCFAs) by digesting dietary fiber, may be associated with lowering inflammatory diseases in humans
- Prevent microbial foes – pathogenic species – from establishing infections
- Many chemicals that are beneficial to human growth and development may be microbial in origin
Disadvantages of Common Human Microbiota

• Some species can produce infections and disease when they become a dominant population.
• One reason for this situation is the over-use of antibiotics and chemotherapeutics to treat infections that also kill many susceptible beneficial species. This upsets the balance of species. Many fungi survive and may cause infections, e.g. the yeast, *Candida*.
• They may produce chemicals that influence our bodily functions in non-beneficial ways.
It’s a Relationship Thing

• The microbiota of our human environment is in intimate relationship with us.
• They are adapted to our bodily temperatures, our organic chemicals, our secretions.
• The microorganisms interact with our food and our secretions, and respond with their own chemical secretions to affect our body.
• The relationship has existed over many thousands of years, and likely even longer.
• Some species of our microbiota can not live anywhere else but on us.
What kind of relationship is it?

• Some species participate in a mutually beneficial relationship called mutualism.
• The human environment provides a home and food for the microbes.
• In return, the microbes produce vitamins, digestive enzymes, and other nutrients that we do not have the genetic capability to produce.
• An example of this mutually beneficial relationship: some gut bacteria produce vitamin K for us.
• Microbiota of the gut are capable of educating our immune system for protection against foes. What about us?
On The Other Hand, It’s not all Equal

• Some microbiota species benefit from the food and temperature and other aspects of our human body.
• But, they do not appear to provide a benefit for us in a commensalistic relationship.
• And, they do not appear to harm us, unless circumstances in the body change.
• This relationship changes when commensals take advantage of environmental changes to cause an infection, they are a pathobiont.
The parasitic relationship – Our Microbial Foes

• Humans are susceptible to diseases from many members of the bacteria and eukarya domains.
• The disease causing organisms are called pathogens, such as the bacterium that causes anthrax, *Bacillus anthracis*, in humans and animals.
• Parasites are often capable of living in the human host and causing harmful effects, and may not cause the death of the host for a long while. An example is the malaria causing parasites, including *Plasmodium vivax*, *P. malariae*, and others.
Helicobacter pylori in stomach, is it a symbiosis that is both bad and good?

- Bad news is association of *H. pylori* in causation of stomach ulcers and cancer
- Good news is that antimicrobial drug treatment works for stomach ulcers in Western medicine
- Does it have a healthy role in young people to train the immune system?
- If it has a healthy role in youth, will antibiotic treatment make it extinct and should we be concerned?
This helix-shaped bacteria called *Helicobacter pylori* is disappearing from our modern stomachs. Once thought of as a “bad bug,” doctors are learning that this bacteria influences our immune system, our weight, and even our height.

Photo courtesy Flickr/AJ Cann
Alimentary Canal and Some Bacteria

Major bacteria present

Enterococci
Lactobacilli

Enterobacteria
Enterococcus faecalis
Bacteroides
Bifidobacterium
Eubacterium
Peptococcus
Peptostreptococcus
Ruminococcus
Clostridia
Lactobacilli
Streptococcus
Staphylococcus

Organ

Stomach
Duodenum
Jejunum
Ileum
Colon
Small intestine
Large intestine

Anus
Obesity and Gut Microbiota

• Obesity is a pro-inflammatory condition associated with a higher population of bacteria of Firmicutes group relative to Bacteroidetes.

• Healthier balances promoting normal weight include a higher ratio of Bacteroidetes to a lower number of Firmicutes type bacteria.

• New research findings find that species of fungi are different in the guts of lean and obese individuals. This may be important to consider as well.
Human Microbiome Through Time

• The specific members of the human microbiome and their population size are influenced by changes:
• in different ages of a human life
• In dietary choices
• Caused by medications including antibiotics
• Rebiosis is the process of consuming probiotics to reverse the imbalance of an environment.
Probiotic Lactobacillli useful in Rebiosis

• Rebiosis is the deliberate application of living beneficial bacteria to an area of the body to repopulate the tissue, improve the balance of species, and prevent damaging inflammation.

• *Lactobacillus acidophilus* and its close relatives are useful for making the vagina more acidic to protect against vaginal infections caused by other species of bacteria.

• *L. plantarum* has been used to prime the respiratory tract to prevent damaging inflammation.

• Also, *L. rhamnosus* primes the nasal tissue against damaging inflammation,
Role of Exercise in Supporting the Microbiome

• Exercise level seems to influence the microbial balance between Bacteroidetes to Firmicutes in the gut.
• Too little exercise may be associated with an unhealthy microbial balance and/or a leaky gut.
• A leaky gut is the condition that allows the exit of microbial chemicals, other chemicals from inside the gut into the human body in concentrations that could enhance inflammation of the human body.
• A moderate level of both our food intake and exercise level appears to help maintain a balanced, anti-inflammatory population of species in our microbiome.
Cholesterol and Cardiovascular Disease: Glossary of Terms

- TMA = Trimethyl amine
- TMAO = Trimethylamine N-oxide
- Foam cells—-inflammatory cells packed with cholesterol
- Arteriosclerosis—arteries become thick and stiff
- Atherosclerosis—build up of fats, cholesterol, etc. in artery walls (plaques)
- Atheroma—plaques in inner lining of arteries
- Atherogenesis—formation of atheromas
Cholesterol Primer

• What is cholesterol and what does it do? Produces cell membranes and creates hormones.
• HDL—”good cholesterol”. Lipoproteins transport it from tissues to liver and bile.
• LDL—”bad cholesterol”. Oxidized form inflames epithelial cell at site of plaque.
• Dietary vs. endogenous cholesterol (liver and cellular function). Dietary only 15%.
• Influx of exogenous cholesterol—from lipoproteins in circulation by desorption or receptor-mediation
• Cholesterol efflux—desorption of plasma membrane, liver and intestine release into circulation
Cholesterol Controversy

• The International Network of Cholesterol Skeptics (THINCS)—Uffe Ravnskov, 2003
• Lipid hypothesis: saturated fats culprit-- lowering LDL cholesterol--statins
• Processed sugars, trans-fats (fries), starchy/processed high glycemic carbohydrates more likely culprits
• CV/Stroke Risk Calculator—9 pieces of info: gender, age, race, total cholesterol, HDL chol., systolic blood pressure, current treatment for high bp, diabetes, smoking
• Statins (7)—simvastatin (Zocor), atorvastatin (Lipitor), rosuvastatin (Crestor) are best
Key colonic bacteria

- Phylum Firmicutes—Most Gram+, round or rod-shaped, most produce endospores. High ratio with obesity.
- Phylum Bacteroidetes—Gram- rods, anaerobic, non-spore forming
- Clostridia—Firmicutes, botulism
- *Proteus*—Gram negative rods, opportunistic pathogens
- *Shigella*—Gram negative rods, facultative anaerobes, *Salmonella*
- *Aerobacter*—Aerobic, divided into *Enterobacter* and *Klebsiella* (diarrhea)
Gut microbiota participates in atherosclerosis in the presence of specific dietary exposures

Dietary sources of phosphatidylcholine

- Carnitine—red meat
- Choline—eggs
- Choline—poultry
- Choline--dairy
- Betaine and choline—Wheaties
- Betaine and choline—Kellogg’s All Bran (high)
- Betaine—beets
- Betaine—spinach
- TMAO itself--seafood
Metabolism of Phosphatidylcholine: Human vs. GI Microbes

• Mammalian Host:
  • dietary phosph’choline
  • choline
  • trimethylamine
  • trimethylamine oxide

• Commensal Microbiota:
  • choline (via GI lumen)
  • trimethylamine (via blood)
Cleveland Clinic and TMAO

- 6/12/13—Stanley Hazen, MD, PhD, Head of Preventive Cardiology and Rehabilitation
- L-carnitine from red meat causes fatty acid translocation into the mitochondria of cells with appropriate gut microbiota
- Gut microbiota increases TMAO
- Increased TMAO increases arteriosclerosis and the risk of heart disease
Hazen—June 2013

- Omnivores have increased capacity to synthesize TMAO from carnitine
- Usually have higher levels of TMAO

- Vegans/vegetarians have decreased capacity to synthesize TMAO from carnitine
Hazen--Phosphatidylcholine

• Dietary choline oxidizes to betaine
• Choline, betaine produce TMAO via gut microbiota and liver enzymes
• TMAO level determined more by gut microbes than diet; other factors include kidney function, genetics, and other microbe exposure
• In mice suppression of gut microbiota inhibits TMAO formation and choline-induced atherosclerosis
Hazen: Cholesterol and TMAO

- TMAO alters cholesterol and sterol metabolism
- Blood macrophages, or foam cells, migrate to sites on blood vessel wall
- TMAO effects cholesterol flux both into and out of blood via liver and intestines resulting in the formation of arterial plaques at those sites
- TMAO blood values predict heart risk better than high blood pressure, cholesterol, and smoking
- Test to measure TMAO levels now available at Cleveland Clinic
Getting Rid of (Harmful) TMAO

• 12/31/15—Dr. Hazen’s mouse study
• Natural substance DMB stops gut bacteria from making TMA
• DMB (3,3-methyl-1-butanol) found in olive and grapeseed oils
• DMB effective way of drugging microbiome to block diet-induced heart disease
• Since not an antibiotic, DMB doesn’t kill “friendly bacteria” and there’s little risk of overusing or building resistance to it
Genetic determinants of flavin monoxygenase 3 expression

- Loscalzo (2011)--
- Many known genetic variants of this mammalian hepatic oxidoreductase
- Wang et al. found correlation of this enzyme with atheroma in mice
- No correlative data yet for human atherosclerosis
Scrutiny of mechanism by which TMAO levels promote atherosclerosis

Loscalzo (2011)—

• Contradictory cellular evidence and the lack of a clear mechanism of action for TMAO
• Further exploration of potential adverse action of TMAO needed
• Possibility TMAO is simply a correlate and not a mechanistic culprit
Effect of related compounds, choline and betaine on atherogenesis

• Both intimately involved in methylation cycle
• Betaine (trimethylglycine) increases methylation potential while suppressing gene expression
• Complex process of genomic methylation could be evaluated in genetically-altered mice
Further Scrutiny of Carnitine-TMAO-Heart Disease

- Koeth et al., 2013 vs. Bill Barrington:
- Choline and betaine, as well as carnitine, convert to TMAO causing atherosclerosis in mice
- Mouse studies used very high doses of free carnitine, choline, and betaine
- Seafood increases blood TMAO, but regular seafood consumption decreases CV risk (numerous studies)
- Small sample size in vegans/vegetarian vs. omnivore study
- Lifestyle may be more important when comparing omnivores to vegans/vegetarians
Gut microbiota participates in atherosclerosis in the presence of specific dietary exposures

Mechanistic link between oral disease and CV disease

- Chao and Zeisel (1990): Formation of TMA from dietary choline.
- *Streptococcus sanguis* and periodontal disease via choline-trimethylamine pathway similar to mechanistic link between gut bacteria and vascular disease.
- Zoeliner (2011) and Fedele (2011) support dental infection and CV disease connection.
Definitions

• Prebiotics: Non-digestible food ingredients that stimulate the growth and/or activity of bacteria in the digestive system in the name of health. E.g., Acacia Gums are richest natural source.

• Probiotics: Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. E.g., Lactobacillus rhamnosus family.
Probiotics and Prebiotics in Cholesterol – Lowering as of 2010

• Some in vivo studies demonstrated effective reduction of serum/plasma cholesterol, LDL-cholesterol, and triglycerides
• Other studies showed insignificant effects on lipid profiles
• Rare cases of probiotic-mediated infections
• Prebiotics generally considered safe, but overconsumption can cause intestinal discomfort
Probiotics

• Supplementing microbial deficiencies by adding bacteria into diet
• Probiotic therapies already used to decrease TMAO levels and reduce atherogenesis in humans (Wang et al., 2011)
• Probiotics lower total and LDL cholesterol due to bacteria binding cholesterol in small intestine preventing blood stream absorption (5/5/14)
Microbiome influences our health

- Other than cardiovascular--
- Asthma—airway immune responses similar to gut resulting in inflammation
- Autism—Gut microbes may relate to brain activity in mice (Rob Knight, Dec. 2013)
- Diabetes / Insulin resistance—Initiates chronic inflammation via LPS (endotoxemia)
- Obesity—Initiates chronic inflammation via LPS
- Irritable Bowel Syndrome—Specific genomic microbiome signatures associated with pediatric IBS