



The Human Microbiome: Germs are Us

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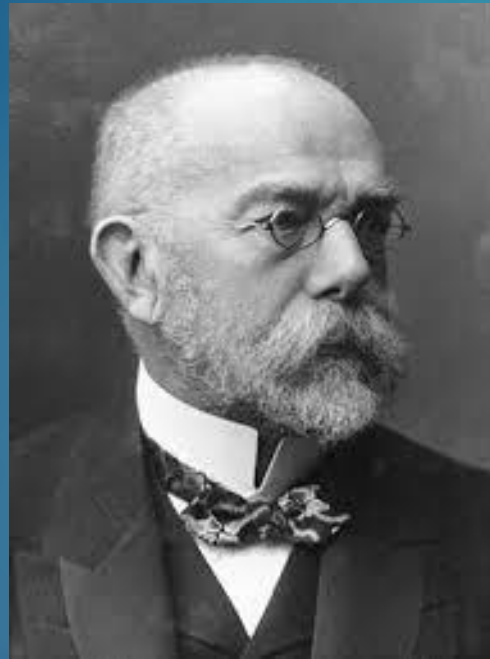




Koch's Postulates:

Proof that a single microbe causes a disease

Robert Koch 1890



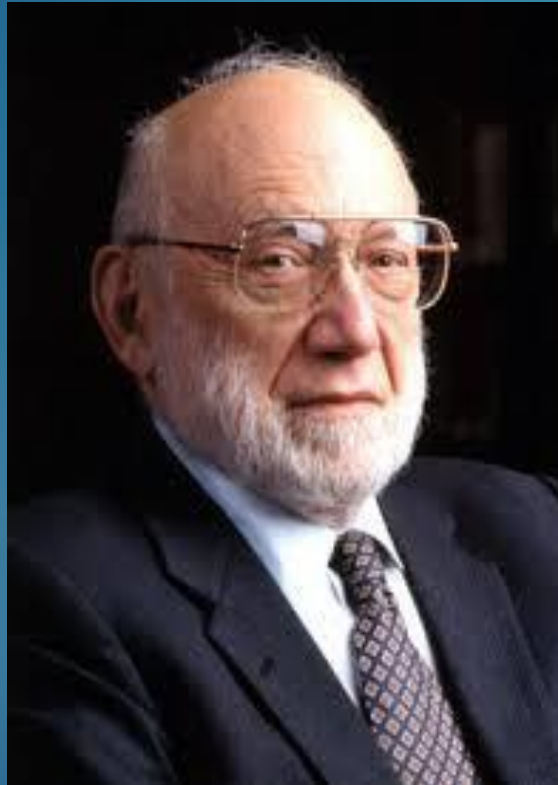
- Isolate single type of microbe from an ill host
- Culture it in the lab
- Infect an animal to reproduce the infection
- Reisolate the microbe from the infected animal

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The Microbiome: Joshua Lederberg



- Microbes can reside together in an environmental niche
- Often in a symbiotic relationship





We are like coral

Dr. David Relman



- We are like coral, a community of diverse life forms living together in an environmental niche





The Human Microbiome:

There are more of them, than there are of us

- 100 trillion bacteria live in and on our body, 10X the number of human cells.
- Half of the stool is not left-over food. It is microbial biomass (2-5 lbs of bacteria).





What are these microbes of our Human Microbiome?

- The problem.....
- <1% of the bacteria grow in culture
- >99% are difficult to identify





The solution: Polymerase Chain Reaction* High throughput gene sequencing

- Don't grow the bugs, grow their DNA by PCR*
 - Match sequences to libraries of known microbial genes to identify bacteria
 - High throughput uses automation as a fast, cheap way to sequence large amounts of DNA
-
- *PCR=Polymerase Chain Reaction





The Human Microbiome Project

Foxman & Rosenthal , Am J Epidemiol 2013;177:197

- 600 normal subjects cultured in 15 or 18 places
- Generated 11,174 cultures
- Genetic material extracted, treated by PCR, sequenced, and identified by matching to libraries of known ribosomal RNA sequences.





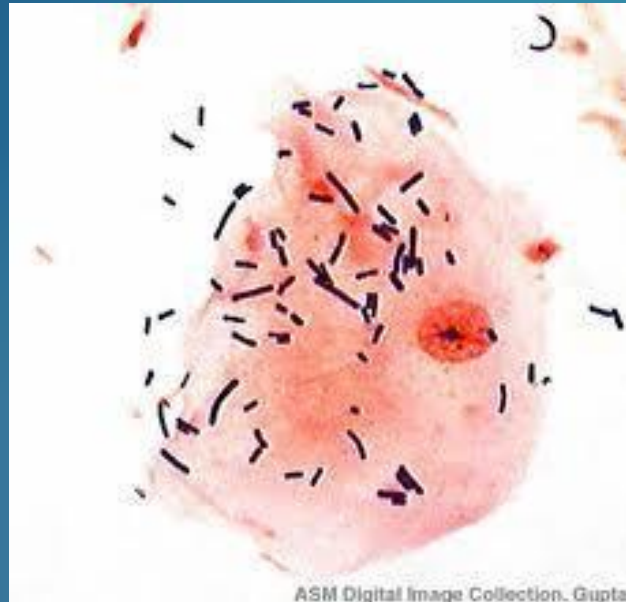
What did the Human Microbiome Project find?

- 1) Our microbiome protects us
- 2) Disruption of our microbiome harms us
- 3) Our microbiome controls our metabolism





1) The Microbiome protects us



ASM Digital Image Collection. Gupta

- Lactobacilli and others protect the vagina, leading to.....
- Healthy pregnancies
- Healthy upper genital tract with reduced risk of STDs*

*STDs-sexually transmitted diseases





2) Disruption of the Microbiome: Bacterial vaginosis

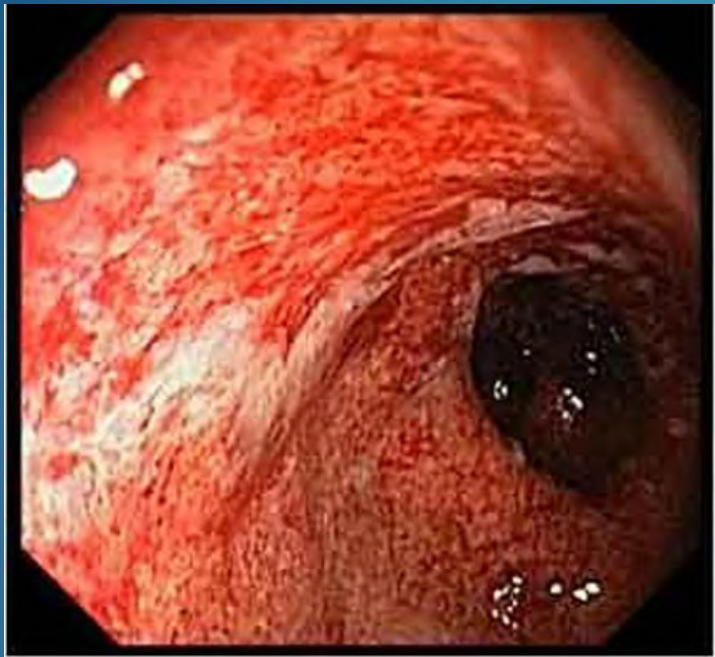


- Loss of lactobacilli creates a void that is filled by anaerobes
 - malodorous
 - Increased risk of HIV, PID and preterm delivery





CDAD: Clostridium difficile associated diarrhea



- Antibiotics disrupt the intestinal microbiome
- Creates a void filled by *C. difficile*
- Abx rx-poor rx
- Fecal microbial transplants (FMT)





Fecal microbial transplant vs vancomycin

van Nood NEJM 2013; 368: 407.

- Randomized trial of frequent relapses of CDAD
- 4 of 13 (31%) were cured by vancomycin
- 13 of 16 patients (81%) were cured by a single fecal microbial transplant





Fecal microbial transplants for relapsing CDAD*

Patel, Mayo Clin Proc 2013; 88:799,

- 30 patients with relapsing CDAD*
 - 29 of 30 (97%) cured with a single fecal microbial transplant
 - Vanco-time to resolution of CDAD-340 days
 - With FMT**-time to resolution of CDAD-3 days
 - Fecal microbial transplant restores the Microbiome
-
- *CDAD-C.difficile associated diarrhea
 - **FMT-fecal microbial transplant

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3) The microbiome controls our metabolism

Turnbaugh, Nature 2006; 444: 1027-1031

- Transplant feces from obese mice to germ-free* mice → mice become obese
- Transplant feces from lean mice to germ-free mice → mice become lean
- Something in the feces was transforming the metabolism of the mice

*gnotobiotic mice were raised in a germ-free environment





Bacteroidetes & Firmicutes in mice

Ridara, Science 2013; 341: 1241214

- 90% of gut bacteria belong to these 2 phyla
- Firmicutes predominate in obese mice.
- Bacteroidetes predominate in lean mice.





Bacteroidetes & Firmicutes in humans

- **When obese humans diet and lose weight, fecal Bacteroidetes increase/ Firmicutes decrease**
- **When lean humans gain weight, Firmicutes increase and Bacteroidetes decreased**
- Was this the cause of weight changes or the result?





Gastric Bypass Surgery

Li, Gut 2011; 60: 1214;

Liou, Science Translational Med 2013; 5: issue 178ra41

- Mice randomized to gastric bypass vs sham surgery
- Gastric bypass mice lost weight.
- Sham surgery mice remained the same weight





Gastric Bypass Surgery

Li, Gut 2011; 60: 1214;

Liou, Science Translational Med 2013; 5: issue 178ra41

- Fecal transplants from each mouse were infused into the intestines of germ-free mice.





Gastric Bypass Surgery

Li, Gut 2011; 60: 1214;

Liou, Science Translational Med 2013; 5: issue 178ra41

- Mice receiving fecal transplants from the gastric bypass mice lost 5% of their body weight .





Gastric Bypass Surgery

Li, Gut 2011; 60: 1214

Liou, Science Translational Med 2013; 5: issue 178ra41

- Mice receiving fecal transplants from the gastric bypass mice lost 5% of their body weight .
- Mice receiving fecal transplants from sham surgery mice didn't lose weight.
- Conclusion: the reason mice lost weight after gastric bypass surgery was not an anatomic malabsorption but an alteration of their gut microbiome.





Kwashiorkor Study from Malawi

Smith, Science 2013; 339:548-554

- 317 pairs of baby twins < 3 yrs old
- 13 pairs became discordant for Kwashiorkor.
- 3 pairs' feces transplanted into germ-free mice.
- Controlled for the presence other intestinal diseases.





Kwashiorkor Study from Malawi

Smith, Science 2013; 339:548-554

- Mice given fecal transplant from twin developing Kwashiorkor, lost weight.
- Mice given fecal transplant from normal twin, did not lose weight.
- Conclusion: disturbance of the microbiome led to the development of Kwashiorkor.





Insulin resistance and the Microbiome

Vrieze, Gastroenterol 2012; 143: 913-6e7

- Men with the Metabolic Syndrome* received gut washout with PEG.**
- Randomized to fecal transplant from lean males or with their own feces.
 - *Metabolic Syndrome-obesity, diabetes, insulin resistance, cholesterol disturbance
 - **PEG- polyethylene glycol





Insulin resistance and the Microbiome

Vrieze, Gastroenterol 2012; 143: 913-6e7

- Fecal transplants from lean donors → increased insulin sensitivity in recipients
- Fecal transplants with their own feces → no change in insulin resistance (controls)





What has the HMP taught us so far?

- How it protects us from infection
 - Impact on obesity and insulin sensitivity in the Metabolic Syndrome*
 - 30,000 new bacterial genes
-
- *Metabolic syndrome-insulin-resistance, diabetes, hypertension, obesity, and dyslipidemia





The Microbiome: many new genes

Qin, Nature 2010; 464:59

- Human genome contains about 20,000 protein-encoding genes.
- Bacteria in our Microbiome possess 100 x as many genes.
- 29,693 new genes found that are unlike any other known genes.
- What do they do?





Muchas gracias Medicina Interna 2017



- “Humans are made up mostly of microbes. We may just serve as packaging.”
--Dr.David Relman





References

- Komaroff AL. NEJM 2017; 317: 355-6. The microbiome and risk for obesity and diabetes.
- Cho and Blaser, Nat Rev Genet 2012; 13: 260-270. The human microbiome at the interface of health and disease.
- Shen et al. Probiotics, Gastroenterol
doi:10.1053/j.gastro.2017.02.003





In a nutshell, a Paradigm shift

- From “One Bug-One Disease” to.....→
- Human health is a collective property of the host and our Microbiome.





Probiotics in CDAD

Johnston, Ma, Goldenberg, Ann Intern Med 2012; 157: 878-888

- Meta-analysis of 20 RCTs, 3818 inpatients receiving antibiotics
- Baseline (placebo) rate of CDAD 5%
- Probiotics-Saccharomyces boulardii, Lactobacilli, Bifidobacteria, Strep sp.
- Probiotics produced a 66% reduction in CDAD compared to placebo





Obesity: a disturbance of microbiome?

Ajslev, Intl J of Obesity 2011; 35: 522

- Antibiotics as growth factors
 - make animals fat.
- Children become obese
 - they receive 10-20 courses of antibiotics
 - esp prone to obesity if <6 months of age
- Childrens' feces
 - Decreased Bacteroidetes and Bifidobacteria
 - Increased Firmicutes





Probiotics: You are what you eat

- Activia yogurt (Dannon)
- Kefir cultured milk (Lifeway)





From probiotics to prebiotics

- We've just spent the better part of a century wrecking our microbiomes with antibiotics, sanitizers, and the Western diet.
- Feed the good bacteria
 - Oligosaccharides for Bifidobacterium
 - Fiber/whole grains for Prevotella





The Human Microbiome: Paradigm shift

- “Humans are made up mostly of microbes. We may just serve as packaging.”
--Dr. David Relman





How does the Microbiome develop?

- Begins at birth from mother's vaginal flora
- Babies born via C-section have different flora
- The microbiome and the immune system develop in concert to age 3.
- Otherwise.....





The microbiome in fat and lean mice

Ley, PNAS 2005; 102:11070

Turnbaugh, Nature 2006; 444: 1027

- Firmicutes predominate in fat mice
- Bacteroidetes predominate in thin mice





The microbiome and diabetes

Perry Nature 2016; 534: 213-7

Devaraj, Clin Chem 2013; 59:617-628

van Olden, Clin Ther 2015; 37: 1172-7

- When Firmicutes predominate vs Bacteroides, influences carbohydrate metabolism resulting in increased acetate production, decreased butyrate.
- Increased acetate → insulin resistance and increased ghrelin (appetite-stimulating hormone and weight gain)
- Increased gut inflammation weakens tight junctions of gut such that endotoxins leak from gut to blood circulation





Lactobacilli make babies thin

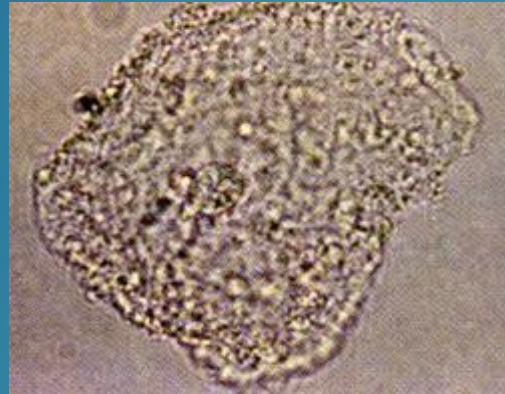
Luoto, Intl J of Obesity 2010; 34: 1531

- 159 Finnish women were randomized to Lactobacilli vs placebo
 - given one month prepartum
 - 6 months postpartum (breast feeding)
 - Lactobacilli given to their babies
- Moderated weight gain through the first 1-2 yrs of life





Bacterial vaginosis: Clue cells



- Biofilms of Gardnerella +
- anaerobic bacteria
 - Mobiluncus
 - Other anaerobes
- PID (pelvic inflammatory disease) due to E.coli and anaerobes



1) Our microbiome protects us

- Normal vaginal microbiome maintains health
 - Lactobacilli protect against disease-causing bacteria in the vagina
 - Loss of lactobacilli creates ecological niche filled by anaerobes (bacterial vaginosis)
 - Treatment with metronidazole poor result





Disruption of microbiome harms us

--Bacterial vaginosis-disruption of vaginal microbiome

--Clostridium difficile colitis-disruption of gut microbiome

- Abx create a void filled by C. difficile
- Treatment with metronidazole poor result
- Fecal transplant: Restoration of normal microbiome





Is obesity a genetic disease?

Radura, Science 2013; 341: 1241214

- Human twin pairs discordant for obesity
- Fecal transplants from fat twin and from lean twin fed to germ-free mice
- Mice fed fat twin feces became fat
- Mice fed lean twin feces remained lean
- Genetics may play at most a minor role in obesity.





Metagenomics identifies bacteria that won't grow in cultures

- “Grow” microbial DNA by PCR*
- Sequence 16S ribosomal RNA genes
- Match to libraries of known microbial genes to identify bacteria that won't grow in culture

*PCR = Polymerase Chain Reaction





The dawn of the antibiotic era

- Alexander Fleming



- Infections of a single microbe are treated with antibiotics





Pasteur and the Germ Theory of Disease

Louis Pasteur



- Microbes are agents of harm and cause disease
- Identify by broth culture





Human Microbiome Project

\$115 million in NIH grants to look at microbes in the gut, nasopharynx, vagina, and on the skin

