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The Microbiome in Inflammatory Bowel Disease and Beyond

Dana Lukin, MD, PhD
Assistant Professor Medicine
Director, Einstein-Montefiore Program for Inflammatory Bowel Disease

- There are aspects of the microbiome relevant to human health and diseases that are connected to them
- Historical Associations: Rene Dubos – began the shift in Germ Theory – from "all bugs are bad" to the first concept that the microbiome is a mutually beneficial component of our bodies and really relevant biologically
- Studying the microbiome comes from human sampling
  - Culture - growing bugs in vitro by collecting species and sequence the gene to figure out the function of those genes. There are so many species in the microbiome, culture is really not practical and often not possible due to various properties of them
  - Metagenomics - harvesting of the DNA to get genetic material (16S ribosomal sequencing) or collect a population analysis to determine which bugs are populating a person's gut. Or you can actually look at the gene product (RNA) and from that can figure out which genes are being expressed, making DNA from RNA to figure out functionally what the bugs are doing and which pathways are being involved
- Factors affecting the microbiome – many things affect the microbiome and starts at birth
- In mammals, Bacteroidetes, Firmicutes are the predominant two (of the 4) phyla members the microbiome; Proteobacteria can be pro-inflammatory bugs
- Bacteria that are in lining of intestine are different than the ones in the lumen; there is a distinct microbiome serving very distinct purposes and a lot of differences of composition in different areas of the body
  - E.g. There is a sterile environment in the stomach, which is excreting HCL 10^{1} organisms/ml compared to 10^{14} in colon demonstrating more diversity as you go into the colon
- Things that influence microbiome now are different than they used to be
  - E.g. In the past vaginal delivery at home used to be the norm and now most children are born in hospitals and increasing rates of cesarean deliveries with less population transfer of microbiome from mother to baby; smaller families, where in the past there were larger families and crowding influenced microbiome; increased use of antibiotics and washing, fewer parasites, low rates of H-pylori in developed cities, refrigeration, eating more processed foods
- Microbiome is very important because it's biologically relevant; Fermentation of food and dietary fiber supply a lot of energy for the body and the different bugs influence different biological functions in the fact that there may be more pro-inflammatory and anti-inflammatory bugs
  - Some compounds fermented are not favorable – ammonia, phenol, etc. and can promote damage of the cells and tight junctions in the gut → leaky gut ad inflammation; Dietary fiber inhibits the fermentation of these adverse products
- What we eat does potentially influence our risk of cancer and possibly other biologically active processes
  - Environmental effects on microbiome: Smokers can have a microbiome that resembles those who are obese or have metabolic syndrome; stopping smoking can promote positive changes to the microbiome and genetic pathways
  - In Africa, extremely low rate of colon cancer compared to African Americans in the US; Study gave Africans traditional Western diet and African Americans controlled African high-fiber diet→2 weeks microbiome makeup changed and resembled the other population
- Inflammatory Bowel Disease (IBD) – series of chronic medical conditions of unknown etiology – mainly Crohn's disease and ulcerative colitis; believed to be inappropriate immune response generated by some trigger (bug, antibiotic, illness, etc.) that can't be turned down by the body causing chronic inflammation and linked to the gut
  - Does the process cause the microbiome degradation or does the microbiome cause the process?
  - There is decreased diversity in microbiome of those with IBD; decreased protective bacteria; increased in proteobacteria causing inflammatory compounds possibly because of impairment of the mucosal barrier
  - Anti-inflammatory metabolites such as short chain fatty acids, tryptophan and indol pathways, which are involved in protecting/decreasing intestinal permeability are decreased in the gut's of patients with IBD→prevents activation in immune-regulatory cells, allowing unregulated immune response to continue
  - Certain bugs that are decreased have been shown to impair regulatory T-cells, which are in place to temper the immune response and dysbiosis can prevent normal down regulation of immune response

- Type 1 and 2 diabetes highly influenced by microbiome
  - Alkanani, 2015: Showed distinct signature of the microbiome in those antibody positive for diabetes vs negative first degree relatives
  - Difference between each of the groups; those with chronic disease is shaping the microbiome over time
- Obesity
  - Ahne et al, 2015: Mice fed either a standard chow diet vs high-fat, high-calorie diet vs high-fat, high-calorie diet with cranberry extract containing phenols (anti-inflammatory); Phenol high diet preventing weight gain and improving energy efficiency in the mice
- Fatty liver disease
  - Hurr and Lee, 2015: Akkermansia in the microbiome correlates with less intestinal inflammation and preventing fatty liver disease; Akkermansia has been shown to be deficient in those with diabetes and metabolic syndrome (high-fat diet); speculation that decreased levels is increasing gut permeability and disrupting tight junctions allowing endotoxins to enter blood stream, associated with insulin resistance and obesity and development of Type 1 diabetes; When dysbiosis is treated with metformin/diabetes meds→ shift to increase Akkermansia in animal models and endotoxin levels in blood decrease
  - Schnabl and Brenner, 2014: examined prebiotics and dietary fibers in diet, which increase short-chain fatty acids; counteracted with high fat diet, demonstrating manipulations of the microbiome manipulate these disease → increases intestinal permeability; inhibits production of lipoprotein lipase and increases free fatty acids to the liver, causing inflammation and levels of FFA to the liver
- Fecal transplant highlights differences in the microbiome of individuals
  - Le Roy et al, 2013: examined response to high fat diet; took stool from mice on high fat diet and put into recipients of regular diet; in responder mice, fasting glucose and fasting insulin levels increased; inflammatory markers increased possibly due to microbiome changes when compared to non-responder mice, demonstrating differences, which may shape our response
  - FATLOSE trial 2012: examined microbiome in those with metabolic syndrome and gave them self fecal transplant vs. transplant from lean donor; patients with lean donor transplant had increased insulin sensitivity and tended towards faster metabolism, demonstrating fecal transfers may influence energy usage in obese patients
- Hypertension: Yang et al, 2015: spontaneously hypertensive rats appears to be a differential microbiome even in hypertension, with narrow diversity
- Other diseases linked to microbiome and changes in intestinal permeability: Rheumatologic disease (arthritis, CD, colitis, psoriasis, etc.); Irritable Bowel Syndrome (a non-inflammatory condition) show decreased diversity and increased access to the gut activate immune cells leading to gut pain; Asthma and allergy linked to low microbiome diversity and early exposure to certain antigens
- Fecal transplant and other therapies
  - First modern use for C. diff; Theorized that some alteration in microbiome predisposes individuals to opportunistic bacteria that “out-competes” other bugs, e.g. chemotherapy proton-pump inhibitors, etc
  - Used in China since 4th century
  - Van Nood et al, 2013: Landmark study; duodenal infusion using NG-tube and effective for patients with refractory C. diff ; meta-analysis of studies demonstrates effectiveness
  - For IBD: Studies terminated for lack of efficacy; magnitude not high enough to justify continuation; Still considering an option of Crohn’s Disease and ulcerative colitis but over time demonstrated response is lost and drift back to pre-existing levels; not sure what’s driving immune pathways; need to examine settings, possibly will be more effective after surgery when there is a “clean slate”
- Prebiotics - dietary fiber that’s able to be fermented to beneficial bacteria; shown in animal models to reduce colitis; however, in humans didn’t show any benefits on disease activity with fructo-oligosaccharides or lactulose
- Probiotics – specific strain demonstrates small benefit in ITT analysis for patients with IBD but not huge magnitude
- Curcumin (turmeric extract) demonstrates some hope for UC; week 4, clinical response 60% vs 12.5 in placebo; subset maintained endoscopic remission

Diet and Gut Microbiota

Tamara Duker Freuman, MS, RD, CDN
East River Gastroenterology & Nutrition

- Dietary influences - The gut microbiota is made not born; as birth process is happening colonization is beginning. How you're colonized and what your colonized with depends on how you're born and over course of first 3 years, very consistent progression of species colonization and is predictable across geographies
  - Vaginal - early colonization is through mother's vaginal microbes; C-section – colonization is through contact with the mothers skin microbes
  - Breast fed infants have higher levels of bifidobacteria; Formula fed infants have a balance between bifidobacteria and other species
  - Other factors: weaning methods (e.g. premastication); what you start eating solid foods are introduced; hygiene, siblings, daycare, pet, urban, rural, antibacterial soap, antibiotic use, etc. → association with allergic disease and asthma
  - Genetic component – children's microbiota resemble that of their parents
- Map of human gut microbiota – 4 predominant phyla
  - Bifidobacteria – typically what is sold in products (yogurt, supplements, etc), however not the highly predominant phylum
- Enterotype – unique to you – describes ecosystem of the bacteria you harbor; Research has landed on 2 or 3 enterotypes
  - Bacteroides – Associated with diets high in animal protein, saturated animal fat, low fiber (Western diet enterotype)
  - Prevotella – Associated with diets high fiber, CHO; more predominant in rural societies and plant based, vegan diets –> good at extracting energy out of fiber
    - Good adaptation in rural place where foods are scarcer and less energy dense; plants are low in calories; Not a helpful adaptation when moving to someplace with a Western diet; mismatch in enterotype with adaptation of new Western diet may explain high rates of obesity and diabetes in these groups
  - Higher ratio of Firmicutes: Bacteroidetes associated with obesity
- Breast feeding – more beneficial enterotype vs formula because of dominance of bifidobacteria; breast milk has indigestible oligosaccharides, which are there to feed gut bugs in baby, nourishing bifidobacteria and have health promoting effects; Also Interaction of breast to mouth provides flora from mothers bodies to promote diversity
- Formula breast milk - getting more sophisticated; new generation formulas with oligosaccharides; seem more likely to be colonized with pathogenic species that are associated with c. diff and e. coli (however no current data looking at rates in adulthood)
- Does method of weaning play a role? Pre-mastication transfer of bacteria in oral cavity; hasn't been studied
  - Hypothesis: timing of exposure for Epstein Barr may plays role in risk of MS risk later in life; when exposed to EB in infancy/toddlerhood may have some effect but not severe; when exposed later in life, may be associated with increased severity (e.g. mono) and risk of MS; pre-mastication may have increased early exposure
- Age 1-3: Start eating solid foods changes evolve towards what gut will look like as an adult by age 3; by this age can't tell between breast feeding and formula fed through stool sample; but may still influence future health risk
- Developing microbiome heavily influenced by diet;
  - Fillippo, 2010: children from Africa and Italy; African children had strains that were absent from western children; bacteria that can break down cellulose and fibrose, which Western children could not break down; microbiota becomes very well adapted based on the diet you have so you're able to extract calories from the food your exposed to
    - 51% Firmicutes, 27% Bacteroidetes (obesity genotype) in Italian children vs 73% Bacteroidetes, 12% Firmicutes in African children; more diversity and different species in African children → more promising in long-term health
  - Another example: Japan – seaweed; can break down agar for food energy – Westerners can't do that
  - Frequency, type, duration, etc. of antibiotic use can have a dramatic effect of microbiota and association with future disease risk, particularly inflammatory bowel disease
  - High fiber diet associated with greater microbial diversity; promotes a healthier enterotype; fiber plays an important energy source for gut bacteria to grow and thrive, and increase short chain fatty acid production
    - Short chain fatty acids have beneficial effects; usable energy source for enterocytes, role in immune regulation, normal cell growth, change pH—acidify colon associated with reduced rates of colon cancer
  - Short term feeding studies – can change predominance of certain species through diet however not much research specifying foods or types of fibers; focus on inulin, chicory root fiber with bifidobacteria and lactobacilli in particular
  - Other dietary factors possible, but not a lot of research yet:
    - Dairy;
    - Iron status–poorly absorbed and travels to colon and bacteria feeds on iron, possibly feeding "bad" bugs (c. diff, salmonella, etc)

    - Malnutrition and nutrient deficiencies – more inflammatory bacteria in people who are malnourished and Iron depleted
    - Exact right balance of nutrients not yet known

- Probiotic Supplements
  - Different strains in probiotics; commercial probiotics are cultured (most gut bugs can't be cultured); single strain vs. multi-strain – we have over 1000 species interacting in complex, symbiotic way; one single strain that has been cultured is likely not the one that's effective for the individual
  - Lack of robust testing on beneficial effect
  - Lax regulation on dietary supplements and quality not tested
  - Health effects are not shown to be significantly strong and not lasting; don't colonize and stay permanently; transient – work as they're passing through the gut but need to keep taking them (when they do have a benefit)
  - Cardioviva / Bile acid hydrolase – shown to be efficacious in lowering cholesterol when taken 2x/day orally by 10%; while significant, 10% of high levels is not really a great change in total numbers and product needs to always be taken
  - Others: Align demonstrated to be beneficial for constipation; VSL for UC, Florastor for C.Diff
  - Risks: Small intestinal bacterial overgrowth (SIBO); In certain circumstances bacteria grows beyond the established threshold (10^5); can cause high levels of discomfort (gassy, bloating, motility problems); over time can cause B12 deficiency
    - One cause is chronic PPI use, which can change pH and be risk factor for overgrowth →probiotic use after using PPI can lead to overgrowth
  - Takeaway: Probiotics on the market today are not really useful
- Microbiota – quite stable – really difficult to change the overall enterotype
  - Change in diet only changes to certain degree
  - If a bug doesn't exist, cant' feed and grow it; need to introduce outside species (eg fecal transplant)
- Care & Feeding of Your Gut
  - Consume the highest FODMAP diet you can tolerate – prebiotic foods in particular (asparagus, artichoke);
  - FODMAP restrictive diets are not for forever; Low FODMAP diet associated with reduced diversity of microbiota
  - Eating food rich in resistant starch – including potatoes cooled in refrigerator, under-ripe bananas
  - Cultured dairy and fermented foods
  - Limiting exposure to unnecessary antibiotics and toothpaste (triclosan)

Speaker Q&A

- Digestive enzymes: can be helpful for digestive tolerance issues; taking the supplementary doesn't really help
    - Need to take enteric-coated
    - Pancreatic deficiency – need to take prescriptive
- Gluten – how does gluten affect the microbiome?
    - Gluten free best suited for celiac disease; however a gluten free diet is also a wheat free diet and influences microbiota
    - Fructans (FODMAP) in wheat play a role; will effect microbiota b/c it's a diet change
- Can a child develop eczema or psoriasis due to c-section at birth?
    - Not likely; It is a multifactorial process that influence it; most development is post-partum (eg IL – 12; IL-23)
- Premastication – are there concerns with dental caries?
    - Dentist don't agree with it; s-mutans can cause tooth decay; little data on whether it plays a helpful role
- What would you recommend someone who finished a course of antibiotic to restore microbiome
    - Data show that at about 3 months microbiota balances out to baseline state; antibiotics suppress gut bugs but don't really sterilize the gut
- Explain cooked grains being cold as beneficial
    - When cooked, simple starch and can break it down enzymatically; when refrigerated the change in temp changes the bond in a way that is resistant to digestive enzymes – it behaves as a fiber and feeds the bacteria (prebiotic effect) – eg. potato salad – cooled ; even if reheated after cooled
- Is there a relationship between intestinal health and PCOS?
    - Not much literature on relationship between PCOS and microbiota – may resemble pre/diabetes but not certain – approach digestive symptoms as would in most patients (fiber supplement, magnesium)
- The gut has been labeled as the 2nd brain; What's the connection with psychiatric disorders
    - Many psychiatric patients have diarrhea – both can be mediated by serotonin; 90% of serotonin receptors are in the gut – the connection may not be exclusive; diet might not address it but medication might.

- What is the connection between social demographic and microbiome?
    - Comprehensive settings between race and class: normal variation still has not been established and have not drilled down to different socio-economic classes; still establishing big broad baseline – individual studies have only looked at individual populations
- What diet would you recommend for acid suppressing meds?
    - There is not a single right diet for everyone; consume healthiest diet they can tolerate and that taste good to them;
    - E.g. GERD patients may have a hard time tolerating raw vegetables
    - Minimal processed food, minimal added sugar
- Are there benefits to apple cider vinegar for GERD?
    - Anecdotally have heard that it may work for some people but vinegar is not well tolerated for many reflux patients