WE HAVE COMPANY: HOW GUT BACTERIA INFLUENCE HEALTH & DISEASE

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The gut environment is complex, with different amounts and types of micro-organisms along its length. What role do commensal intestinal micro-organisms have on homeostatic mechanisms in health and development of disease? This article explores some of these emerging frontiers in our understanding of gut bacteria.

Discoveries made in the last five years or so have shown that we are introduced to micro-organisms even before our birth. Contrary to earlier belief that the mother's womb protects a developing foetus against micro-organisms, we now know that bacteria from the mother's blood can enter the amniotic fluid (that surrounds the developing foetus). The type and numbers of these bacteria have a huge bearing not only on pregnancy outcomes but also on the immune system of the new-born. Our association with microbes becomes more rapid at the time of our birth. A new-born is exposed to micro-organisms in almost everything it makes contact with – its mother's birth canal, skin and breast-milk; other food; and the environment. All its exposed surfaces, including its skin, eyes, ears, reproductive tract and gut, are quickly colonised by microbes. The nature and size of these initial microbial populations can vary initially with changes in diet and environment, but as a child grows older, these microbial communities become more stable in composition. The gut of an adult has over thousands of species of micro-organisms of all kinds – bacteria, fungi and viruses, and contributes nearly 2 kilograms of her body weight!

> **Colonisation:** This is the process by which microbial cells multiply to establish a 'colony' after entry into the body. Usually, different microbial species abound in different parts of the body - bacteria on teeth surface are different than those on the cheek, which in turn differ from those colonising the tongue, etc.

Since Antonie von Leeuwenhoek published his work in the early 1700s, scientists have known that micro-organisms are found at various sites in the human body, particularly the gut. We've also known that not all microbes that are found at a particular location are permanent residents; some only appear transiently. Those that are permanent residents establish long-term interactions with their host, forming stable populations that may have demonstrable functions.

Recent research has begun to demonstrate just how complex some of the interactions between

Antonie von Leeuwenhoek: Widely considered to be the 'Father of Microbiology', Leeuwenhoek was, in many ways, an unlikely scientist. A tradesman by profession, he had no money, no degree; and the only language he knew was his native - Dutch. Despite these disadvantages, he made some of the most seminal discoveries in Biology. Grinding lenses, he made simple microscopes — 500 of them over his lifetime! He discovered sperm cells, blood cells, bacterial cells on teeth scrapings, as well as microscopic rotifers and protists.

microorganisms and their host **really** are. Improvements in laboratory science and (animal) model systems are helping us understand some of the mechanisms by which human–microbial interactions occur, and how they influence health and disease in their human hosts.

How are intestinal microorganisms studied?

Understanding our relationship with intestinal micro-organisms depends upon identifying the types and numbers of the different micro-organisms in the gut; and the nature of interactions between different micro-organisms, as well as between micro-organisms and our bodies.

The conventional approach to doing this would involve isolating individual microbial species from the gut, and inducing them to reproduce under controlled laboratory conditions (popularly called microbial culture, or simply 'culture'). A variety of physico-chemical and biological tests would then be used to uniquely identify these colonies, in a process that is somewhat similar to identifying human beings based on their facial features. This approach has limited uses, however, because we have not been able to grow the many microbes that thrive in the low- (or no-) oxygen environment of the human gut under laboratory conditions.

Microbiota

This term refers to the collection of micro-organisms that are found at a particular location, for example, the skin of an individual or an ocean vent.

In contrast, recent advances in genetics have made it possible to identify these micro-organisms by their nucleic acids in a process akin to fingerprinting in humans. Also, gnotobiotic, knock-out, transgenic and humanized mice models are helping us decipher the cross-talk between gut microbes and their human hosts.

Animal models in characterising gut microbiota

Gnotobiotic (from Greek *gnostos*, known; and *bios* meaning life) refers to a laboratory animal, commonly a mouse, whose microbial composition is known in its entirety. These animals are raised in germ-free environments, and then introduced to one or a few species of micro-organisms in a controlled manner under laboratory conditions. The effects of this specific kind of colonization on the gnotobiotic animal are used to understand similar interactions in humans or other hosts of the microbes.

Knock-out is a laboratory-raised mouse in which a specific gene has been inactivated, or 'knocked out'. Evidence suggests that the genetic background of individuals – the presence/absence of certain genes - influences the composition of their gut microbiota. Scientists can investigate this by evaluating the effects of knocking-out the mouse counterparts of specific human genes on the composition of their gut microbial communities.

Transgenic is any plant or animal with gene(s) from another organism inserted into its genome. This kind of insertion can either happen naturally (for example, genes from disease-causing bacteria found inserted in the DNA of hundreds of varieties of sweet potato), or by deliberate human intervention.

Humanised mice are mice with transplanted human faecal microbiota in their guts. These are compared with normal mice to identify the role of intestinal micro-organisms in human metabolism.

Thus, information is now emerging not only on 'who is there' in the gut, but also on 'what are they doing?' With an improvement of methods to study them, we are now able to characterise not just the diversity of microorganisms in the gut, but also their interactions and functional stability.

Microbial communities in the human gut

Much of our early information on colonisation came from studies of aerobic and anaerobic bacteria that could be cultured in labs. These studies have shown us that the human gut is differentially colonized, with the number and diversity of bacteria in it increasing

progressively. Thus, for every milliliter of its contents, the stomach has ~10,000 bacterial cells, the small intestine (ileum) has substantially higher density than this (~108 bacterial cells/ml), and the distal colon houses even greater numbers, ~1013 bacteria/ml. To add to this complexity, different bacterial species dominate different locations of the gut, with *Helicobacter* spp. found in the stomach, facultative anaerobes and strict anaerobes in the ileum, and predominantly anaerobic bacteria in the distal colon. What's more – the composition of microbial communities in the human gut can vary over the lifetime of an individual and up to as much as 30% between individuals.

Based on the composition of their gut microbiota, a research study in 2011 classified all humans into three categories or 'entero'-types. Humans belonging to the first category, and called Type I entero-types, have high levels of *Bacteroides* species, and thus also of the enzymes for synthesis of biotin (Vitamin B7) that these bacteria produce. Type 2 entero-types have less *Bacteroides* spp. but more *Prevotella* spp., and, therefore, more enzymes for thiamine (vitamin B1); while Type 3 have high levels of *Ruminococcus* spp.

Diet and the intestinal microbiota: you are what you eat

The relationship between intestinal microbiota and their host is strongly influenced by the diet of the host.

By acting as substrates for microbial metabolism, the nutrients we consume play an important role in altering the structure of microbial communities in the gut. One example of this is seen in the fact that when compared to formula-fed infants, breastfed ones show higher levels of bifidobacteria. Bifidobacteria have multiple health-related benefits in a newborn, such as the protection of the gut mucosa, increased production of immunoglobulin A, and the ability to metabolise carbohydrates in breast milk.

Similarly, another study compared the intestinal microbiota of children in rural Burkina Faso in Africa, with children in Europe. While the diet of the African children was rich in complex carbohydrates, fibre and non-animal protein, that of their European counterparts was rich in animal protein, sugar, starch and fat. This study showed that children in Burkina Faso had greater microbial richness, more *Prevotella* and less *Bacteroides*, and produced higher levels of short-chain fatty acids than children in Europe. Other studies have shown that greater microbial richness is associated with diets higher in fruits, vegetables and fibre; while lower richness is associated with multiple diseases — obesity, insulin resistance, dyslipidemia (abnormal amounts of lipids in blood) and inflammatory disorders.

Apart from influencing their composition and/or richness, diets can also alter the metabolic functions of intestinal microbial communities in humans. Intestinal bacteria digest many types of food to produce small molecules that are then metabolised in the human liver, and have an important role in human physiology. For example, carbohydrates in starch can be broken down by colonic bacteria to produce short-chain fatty acids, which regulate several functions related to immunity and lipid synthesis.

Intestinal microbiota in health and disease

Although there are differences between the intestinal microbiota of different individuals, in general more than one microbial species can perform a single metabolic function in the human body. This means that in spite of differences and changes in the number, type and proportions of individual microbial species, their human hosts can continue to have normal gut function.

Gut microbiota and the host adapt to each other through some very interesting evolutionary and molecular processes. One example of this is seen among the Japanese. Sushi, a dish made by wrapping rice and raw fish in nori (derived from seaweed), is an important part of the Japanese diet. Nori is the only food humans eat which contains a special class of complex carbohydrates called porphyrans. We know of only two organisms with enzymes, called porphyranases, capable of breaking down porphyrans. One of these is a marine bacterium, called *Zobellia galactonivorans*, which naturally grows on seaweed. The other is a gut bacterium, called *Bacteroides plebeius*, found only in the intestines of the Japanese. It seems likely that this human gut bacterium acquired the genes for porphyranases from *Zobellia* ingested along with the seaweed that forms part of the regular diet of the Japanese. By acquiring these genes, gut bacteria are able to break down the carbohydrates of the seaweed, thus exploiting an additional source of energy.

Useful Websites

http://academy.asm.org/index.php/faq-series/5122-humanmicrobiome

http://www.gutmicrobiotawatch.org/en/gut-microbiota-info/

Extensive research on the microbiota shows that intestinal bacteria modulate genes involved in several different intestinal functions in humans, including nutrient absorption, carbohydrate metabolism and intestinal motility.

Preventing infection: The barrier function

One of the most significant benefits we derive from resident gut microbiota is that these microbes act as a defensive barrier against potential pathogens. They do this through a variety of mechanisms, one of which involves the production of antimicrobial substances that are active against several intestinal pathogens. For example, *Lactobacillus* and *Bifidobacterium* spp. produce antibacterial substances active against a wide range of pathogens including entero-pathogenic *E. coli*, and *Listeria monocytogenes*, etc. Other mechanisms used by gut bacteria to prevent pathogen colonization include impairment of flagellar motility and prevention of cellular damage in the host.

Figure 1. An electron micrograph of a cluster of *E. coli*, magnified 10,000 times. Source: Photo by Eric Erbe, digital colorization by Christopher Pooley, both of USDA, ARS, EMU, Wikimedia Commons. License: Public Domain. URL: https://en.wikipedia.org/wiki/File:E_coli_at_10000x,_original.jpg

Nutrient uptake

One of the most exciting findings about the host-microbe relationship in recent years has been the role of the intestinal microbiota in malnutrition.

The relationship between the microbial flora of the gut and the host depends on the capability of either or both partners to utilize nutrients in human diets. Does this mean that gut microbes actively compete with us for nutrients from our food? On the contrary, experiments comparing the calorie intake of conventional and germ-free mice show that the former require 30% less calories to maintain their body weight. This indicates that gut microbes help us derive the maximum nutritional value from available nutrients.

Several studies link gut microbiota to obesity and malnutrition. In one of these studies, germ-free mice put on weight when they were transplanted with gut microbes from an obese person, but not those from a thin person. It was also possible to use the gut microbiota from a thin person to displace the microbiota of obese mice. As long as they were put on a healthy diet, this transplantation could prevent the obese mice from gaining weight. In another study, faecal samples were collected over a long period of time from twins in Malawi, a landlocked country in south-eastern Africa that has one of the highest infant mortality rates in the world. Results from this study, published in 2013, showed that the gut microbiota of children suffering from a severe form of malnutrition, called Kwashiorkar, was very different from that of unaffected peers of the same age-group. When the microbiota of the malnourished children was transplanted into gnotobiotic mice fed with Malawian diets, they lost weight, and showed an altered amino acid and carbohydrate metabolism. A similar study by the same group, conducted in Bangladesh, showed that the gut microbiota of malnourished individuals were typically like that of much younger individuals, or 'immature'. Symptoms of malnutrition could be reduced slightly by therapeutic feeding, with malnourished individuals showing temporary maturation of their gut microbiota, but soon reverted to their previous 'malnourished' state. Studies to explore whether therapeutic food could lead to a more permanent maturation in gut microbiota are currently underway.

With diets that primarily consist of carbohydrates, humans are well-equipped to digest disaccharides, as well as absorb the monosaccharides produced as a result. However, our capacity to hydrolyse and utilise other complex polysaccharides, particularly those of plant origin such as cellulose, xylan and pectin, is limited. These carbohydrates can be digested by some anaerobic bacteria that reside in in our distal colon. Equipped with specific enzymes for degradation of complex polysaccharides, these microbes break down undigested dietary carbohydrates to short chain fatty acids that can then be utilized by different organs of our body. Thus, commensalism or 'eating together' helps gut microbial communities derive energy from us, while we benefit from this association by utilizing otherwise indigestible carbohydrates.

Disease states and the microbiota

While several studies have shown that a new-born's immune system co-evolves with the microbial community in her gut, gut microbiota may also contribute to some diseases, like atherosclerosis (or a hardening of arteries), by breaking down dietary lipids to harmful metabolites. Similarly, gut microbes have been shown to break down another dietary component – choline, to produce trimethylamine oxide, a small molecule that is strongly associated with an increased risk for coronary vascular disease in humans.

There is evidence that the gut microbiota also contributes to several forms of liver disease including non-alcoholic fatty liver disease, alcoholic and auto-immune liver disease. Similar associations between altered gut microbiota and disease states are also found in inflammatory bowel disease, diabetes and colon cancer. The mechanisms by which gut microbes cause these different diseases are still being investigated.

Using microbiota for treatment - faecal microbe transplants

While they can contribute to diseases in the host, gut microbes can also be used to cure certain ailments.

Clostridium difficile is an anaerobic bacterium that is commonly found in the gut. However, the use of antibiotics or a severe inflammation of the large bowel in hospitalised patients can lead to an overgrowth of *C. difficile*. This can result in severe diarrhoea (up to 15 times a day), abdominal pain, weight loss, fever, and even be fatal. Even when treatment with antibiotics is initially successful, the infection can recur. This is believed to be due to the inability of the intestinal microbiota to suppress the growth of the toxin-producing *C. difficile*.

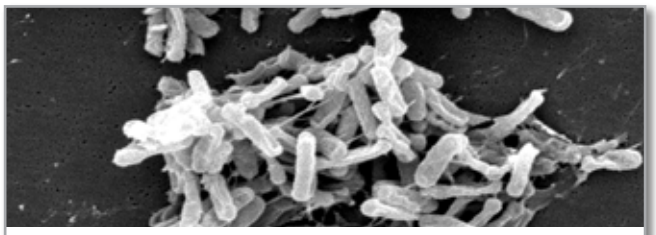

Figure 2. Scanning electron micrograph of *Clostridium difficile* from a stool sample. Source: CDC/ Lois S. Wiggs (PHIL #6260), 2004, Obtained from the CDC Public Health Image Library, Wikimedia Commons. License: Public Domain. URL: https://en.wikipedia.org/wiki/Clostridium#/media/File:Clostridium_difficile_01.jpg

Recently, Faecal Microbiota Transplant (FMT) has begun to be used to treat this recurrent condition with some success. FMT is a procedure in which faecal matter, or stool, is collected from a tested donor, mixed with a saline solution, and placed in a patient's gut through an enema or an endoscopy. Faecal transplants have also had good results with other digestive or auto-immune diseases, including Irritable Bowel Syndrome and inflammatory bowel disorders.

Conclusion

In the past decade, there has been increasing interest in the human microbiota, and new methods and tools are being developed to enable more detailed studies. One important reason for this interest has been the recognition that the commensal microorganisms comprising human microbiota are more than just passengers in the host, and may actually regulate certain vital host functions. With a better understanding of the contribution of intestinal microbiota to specific disease states, it may be possible to develop new strategies or drugs to modulate these microbes to treat or prevent diseases.

References

1. Arumugam M, Raes J, Pelletier E et al. Enterotypes of the human gut microbiome. Nature 201; 473(7346):174-80.
2. Riduaura VK, Faith JJ, Rey FE et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013;341(6150):1241214
3. Biteen JS, Blainey PC, Cardon ZG et al, Tools for the microbiome: Nano and beyond. ACS Nano. 2015 Dec 22. [Epub ahead of print]
4. De Filippo c, Cavalieri D, Di Paola M et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A. 2010; 107(33):14691-6.
5. Hehemann JH, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. Nature. 2010;464(7290):908-12
6. Smith MI, Yatsunenko T, Manary MJ et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science. 2013; 339(6119):548-54.
7. Ierardi E, Sorrentino C, Principi M, Giorgio F, Losurdo G, Di Leo A. Intestinal microbial metabolism of phosphatidylcholine: a novel insight in the cardiovascular risk scenario. Hepatobiliary Surg Nutr. 2015; 4(4):289-92.
8. Kelly CR, Kahn S, Kashyap P et al. Update on fecal microbiota transplantation 2015: Indications, methodologies, mechanisms and outlook. Gastroenterology. 2015; 149(1):223-37.

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