The changing hypothesis of the gut

The intestinal microbiome is increasingly seen as vital to human health

Philip Hunter

In many ways, humankind's story of the past two centuries is that of a battle against dirt. From the development of the germ theory of disease to the modernization of sewerage systems in large cities, our growing awareness of the microorganisms and diseases that come with dirt has led to better public health and better hygiene. Yet, despite the increased lifespans and reduced infant mortality that have partly resulted from such improvements, the medical establishment has seen an increase in allergies and autoimmune diseases in the industrialized world in recent decades.

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Several hypotheses have been put forward to explain the trend, ranging from exposure to environmental contaminants to changing diets. Yet none so far have given an entirely satisfactory answer. One of the most interesting suggestions that has enjoyed some popularity, but has never caught on with many researchers or clinicians, is the hygiene hypothesis, which suggests that humanity's new-found cleanliness is itself the problem. The hypothesis, which was first proposed more than two decades ago [1], posits that childhood exposure to pathogenic organisms, especially certain bacteria, is essential for training the immune system to become tolerant of the many neutral or benevolent strains of microbiota that enter or reside in our bodies; without this exposure, the immune system overreacts to environmental cues. The problem with the

hypothesis, though, is not that it is demonstrably wrong, but that it remains incomplete and has so far lacked hard evidence of a link between exposure to pathogens and specific immunological mechanisms.

In the post-genomic era, however, a new and more complete theory has emerged to explain the rise in allergic and autoimmune diseases. The 'disappearing microbiota' hypothesis does not point the finger at any single aspect of modern life, but suggests instead that some—if not all—developments over the past century, such as clean water, modern birth practices, pollution and the increasing use of antibiotics, have all contributed to a shift in the balance between different species and types of microorganism in the gut. This shift has, in turn, altered our symbiotic relationship with our gut microflora and the health benefits that our tiny passengers have conferred on us in the past.

The disappearing microbiota theory has emerged with our growing knowledge of the importance of the microbiome in immunity and health [2]. One important implication is that changes in the composition of the microbiome, at the population level, must have consequences—potentially both positive and negative—and that these must be taken account of in health policy. "We and others have proposed the microbiota hypothesis," said Sarkis Mazmanian, an Assistant Professor in the Division of Biology at the California Institute of Technology and a specialist in the evolutionary mechanisms of host-bacterial symbiosis. "It is not reduced infections that are mediating increases in allergic and autoimmune disease, as proposed by the hygiene hypothesis, but the lack of exposure to gut bacteria, as in the microbiota hypothesis" [3].

According to Martin Blaser from the New York University School of Medicine, well known for his studies of the link between *Helicobacter pylori* and human diseases, this lack of exposure to gut bacteria is leading to a gradual shrinking of the human microbiome, at least in affluent nations. "This is the disappearing microbiota hypothesis," Blaser said. "I came to this hypothesis through my work on *Helicobacter*, which is clearly disappearing. But the disappearance seems to have begun even before *Helicobacter* was discovered, and not because people are treating ulcers," he explained, referring to the common practice of treating stomach ulcers with antibiotics designed to kill *Helicobacter*, following the discovery of its causal link with the condition [3].

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In fact, Blaser suggests that various factors are involved, including the overuse of antibiotics, as well as the chlorination of drinking water. "We know that chlorination of water impedes the spread of pathogens, but another thought is that it impedes the spread of commensals," he explained. But Blaser does not think we should stop chlorinating water, nor does he want to turn back the clock on antibiotics. Even so, he does make an important point: "Antibiotics are wonder drugs, but everyone assumed they would be free, with no biological cost. When you start learning about our microbiome, it's not too hard to imagine courses of antibiotics leading to extinctions, and when

[the commensals] are gone, they're gone. It was assumed that everything bounces back when the course is over, but there is more and more evidence that this is not the case." Indeed, Blaser believes that the microbiome is gradually disappearing in terms of the total number of species present, but that this is hard to spot when there are so many and their numbers are so variable between individuals. "If you have thousands of species, you may not see it at first, but our hypothesis is that it is cumulative," he explained.

Regardless of whether the microbiome is shrinking in diversity, the idea that it is profoundly important in human health is gaining credibility, most immediately in inflammatory conditions directly relating to the gut, such as irritable bowel syndrome (IBS). Virtually nobody in the field disputes the idea that the microbiome is implicated in IBS, but it is less clear whether it is a cause, an effect, or a combination of both. The question is whether conditions such as IBS are caused by *a priori* changes in

the microbiome, or whether such changes are symptoms of diagnostic value.

The latest evidence that the microbiota have a protective effect in the gut comes from a recent study using mice [5]. Richard Flavell and colleagues at the Yale School of Medicine found that deficiencies in the NLRP6 inflammasome, engineered by a deletion mutation, resulted in an imbalance in the gut microbiota. The new microbiota population was colitogenic and led to inflammation of the colon. "But more importantly, we found that this pro-colitogenic flora was transmissible to wild-type mice that were co-housed with NLRP6 inflammasome-deficient mice, and induced exacerbated inflammatory bowel disease (IBD) in both NLRP6-deficient and co-housed wild-type mice," Flavell explained.

In other words, IBD can be transmitted to healthy animals that are not NLRP6 deficient, possibly through the spread of commensal bacteria or their products, such as metabolites, that might induce a change in the microflora. As Flavell indicated, although

the NLRP6 inflammasome is known broadly to regulate the composition of the microbiota through release of the pro-inflammatory signalling cytokine interleukin-18 (IL-18), his latest work raises questions over the specifics of this process. "What types of microbial components or metabolites activate the NLRP6 inflammasome and how IL-18 regulates the ecology of the gut microbiota are important questions that remain to be resolved in the near future," he said.

This finding that metabolic conditions can be transferred to otherwise-healthy individuals should be followed up to see if it can be replicated in other animal models and ultimately in humans. This is of particular interest, given that the causes for the recent near-epidemic of obesity among some population groups have yet to be fully explained.

Perhaps more probable, however, is that a change in the microbiome follows the onset of a condition such as obesity, rather than being the underlying cause. Even so, it might still be possible to reverse

the syndrome by forcing a change in the composition of commensal bacteria in the gut. Work by Willem de Vos and colleagues at the Laboratory of Microbiology, Wageningen University, the Netherlands, is examining the potential for prebiotic and probiotic therapies that can be tested on humans, partly on existing published data from human and animal research [6].

Another important indication emerging from the work of Flavell, de Vos and others is that the influence of the microbiome extends beyond metabolic disorders, for which few dispute the importance of commensal bacteria, to include disorders affecting a variety of organs and systems elsewhere in the body. In the case of some inflammatory liver diseases, this link was already known. There is a strong association, for example, between inflammatory gastrointestinal diseases—such as ulcerative colitis and the chronic liver disease primary sclerosing cholangitis, which involves scarring of the bile ducts of the liver [7]—and the presence of commensal bacteria. Flavell and colleagues found that dysbiosis associated with deficiency in the inflammasome regulates hepatic inflammatory processes in non-alcoholic fatty liver disease [8], which is highly prevalent in western societies. This study found that wild-type mice co-housed with inflammasome-deficient mice developed exacerbated steatohepatitis—a type of liver disease—as well as obesity, suggesting there is also a contagious element to some liver diseases.

The liver is anatomically close to the intestines, but there is growing evidence that the influence of the microbiome permeates organs and systems elsewhere in the body, including the central nervous system. Studies have evaluated evidence for connections between the microbiome and multiple sclerosis, for example, suggesting that it could have potential both for diagnosis and therapies in future. Glenn Gibson, Professor of Food Microbial Sciences at the University of Reading in the UK, commented that such findings are “not too surprising because the metabolic output of gut bacteria is massive and bound to exert effects locally as well as systemically.” He explained that the effects “can be positive or negative for health, depending on which bugs and which metabolites are involved. But the really great news is that we are able to alter the situation to improve things and affect health. Unlike our genetics,

the gut microbiome can be changed.” If, as Gibson suggests, the gut microbiome is responsible for about 70% of the total immune response, this could have profound consequences for the treatment of disease.

However, for treatments based on probiotics or metabolites derived from bacteria to become widely available, there will have to be a marked shift in the attitude of some regulators, according to Gregor Reid, Chair in Human Microbiology and Probiotics at the Lawson Health Research Institute in London, Ontario, Canada. Reid and colleagues have conducted trials on the use of probiotic lactobacilli, a main component of the lactic acid bacteria group, to improve the treatment of vulvovaginal candidiasis (VVC) [9]. This is a condition caused by a strain of yeast that affects around 75% of sexually active women at some stage of their lives, causing vaginal itching and discharge. Reid was incensed when the European Food Safety Authority (EFSA) refused to approve this probiotic treatment for VVC in the European Union, leading to his publication of an opinion paper countering the EFSA critique [10]. “In this recent EFSA ruling, they stupidly disassociated nutrition from vaginal health, when in fact it is critical,” Reid explained.

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At least this incident has highlighted issues relating to the use of, and approval for, treatments that attempt to alleviate conditions through manipulation of the microbiome. In particular, it highlights the need to nail down direct molecular associations between components of the human microbiome and specific cells or systems in the body that underpin conditions such as VVC. This is a main focus of research at the Functionality of the Intestinal Ecosystem (FinE) lab at the Micalis Institute in Paris, France. “The major priority of my own research team is to use functional metagenomics to identify signal molecules and crosstalk mechanisms linking human intestinal commensals and human cells using *in vitro* high throughput phenotyping systems,” explained Joël Doré, Vice Head of FinE. “The basic concept is that intestinal commensals constantly exchange signals with human cells, including intestinal epithelial cells, immune cells and even distally

located peripheral tissues from adipose tissue to liver to brain.”

It is probable that new diagnostic approaches will emerge from such metagenomic work on commensals even before treatments, which require a greater burden of proof and longer cycles of approval. It is not just regulators, but pharmaceutical companies that will have to embrace the idea of probiotics and metabolites before treatments become widely available in mainstream health care. There is great optimism from researchers in the field, but they still have to convince regulators and big pharma companies that the microbiome will become a main source of new therapies.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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Philip Hunter is a freelance journalist in London, UK.

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