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LARGE INTESTINE (B CASH, SECTION EDITOR)

Progress in Our Understanding of the Gut Microbiome: Implications for the Clinician

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Abstract The investigation of the role of the microbial communities of our gastrointestinal tract (microbiota) has accelerated dramatically in recent years thanks to rapid developments in the technologies that allow us to fully enumerate and evaluate the full complement of bacterial species and strains that normally inhabit the gut. Laboratory studies in a range of inventive animal models continue to provide insights into the role of the microbiota in health and to generate plausible hypotheses relating to its potential involvement in the pathogenesis of human disease. Studies of the composition of human gut microbiota continue to accumulate but their interpretation needs to be tempered by an appreciation of the limitations of single-point-in-time studies of fecal samples from small study populations. Nevertheless, clinically important examples of a central role for microbiota-host interactions in disease pathogenesis have emerged and many more have been postulated but await confirmation in appropriately powered and conducted studies.

Keywords Microbiome · Microbiota · Bacteria · Antibiotic-associated diarrhea · *Clostridium difficile* · Inflammatory bowel disease · Irritable bowel syndrome · Liver disease · Colorectal cancer

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Introduction

In coining the phrase "you are what you eat," Anthelme Brillat-Savarin, the French gastronome, was not to know that interactions between food, the gut microbiome, and the host would come to achieve such a pivotal role in health and disease. While the central role of food ingestion on the stimulation of a host of physiological functions of the gastrointestinal tract (from motility to secretion) has been known for at least a century, the complexity and range of interactions between dietary components and the gut microbiome have only begun to be appreciated recently [1••]. Much of this progress has been facilitated by the rapid evolution of the technologies that allow us to document in detail the components of the microbiome, their genetic composition, and metabolic potential [2]. Applying these techniques, symbiotic relationships between our bacterial fellow travelers and our immune, metabolic, and neuroendocrine systems, to name but a few, have been identified and the consequences of a breakdown in such mutual interdependence in terms of disease causation increasingly revealed. Before we move to discuss the ramifications of the "hottest" area of biomedical science for clinical practice, let us pause for a moment to familiarize ourselves with the lexicon that permeates this literature.

Terminology

The term microbiota refers to the totality of microorganisms in a given environment, such as the gut, and includes not just bacteria but also archaea, viruses, fungi, and others. For this reason, the term microbiota is now preferred to "flora."

Strictly speaking, the term microbiome refers to the entire habitat: i.e., the totality of microorganisms in a given environment together with their collective genetic material and the

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surrounding environmental conditions. However, even in the microbiological literature, the terms microbiome and microbiota are often used interchangeably to refer to the totality of microorganisms.

The term metagenome refers to the collection of genomes and genes from the members of a microbiota and the metabolome to its metabolic products.

While the composition of the microbiota can be identified using high-throughput sequencing techniques based on the analysis of 16S rRNA gene sequences amplified from a given environment, the complete enumeration of bacterial genomes necessitates the utilization of quite different methods, such as shotgun sequencing [2, 3•, 4, 5]. Computational methods and skilled analysis with application of powerful algorithms are then used to annotate genes [6]. This information provides a more comprehensive understanding of the potential functions of, and interaction between, microbial communities [7•].

The Gut Microbiota in Health: Regulation and Impact

The microenvironment of the gastrointestinal tract is greatly influenced by such obvious phenomena as diet and antibiotic use as well as host factors such as the presence of inflammation or disease, and a multitude of other environmental factors (both macro and micro) that are still being elucidated [8]. That such diversity in the composition of the gut microbiota should exist between individuals should come as no surprise. However, while Karlsson and colleagues observed that differences in species, gene richness, and diversity existed across populations, a common ("core") gut microbiome is shared, represented by approximately half a million microbial genes, between individuals [9]. Similarly, interrogation of the large data produced by the European microbiome consortium revealed that their population could be divided into three subpopulations (which they referred to as enterotypes) based on the dominance of a specific species; a segregation that seemed to be driven by dietary patterns [10].

Some of the cardinal influences on the composition of the microbiota were revealed in a seminal study on individuals who ranged in age from birth to 70 years in an Amazonian Indian population in Venezuela, rural Africans in Malawi, and urban dwellers in the USA that found the overall composition of the gut microbiota of each population to be quite distinct with unique patterns of over- and under-repressed genes between populations [11]. Across all these diverse populations, a clear longitudinal trend was evident: the gastrointestinal tract of the infant is virtually sterile at birth and rapidly increases in terms of both bacterial numbers and diversity thereafter [11]. The infant gut is first colonized by maternal and environmental bacteria during delivery and continues to be populated depending upon mode of delivery (vaginal birth vs. cesarean section), diet (breast milk vs. formula), level of sanitation, exposure to vaccinations, and other contacts [12, 13]. By 2 to 3 years of age, the child's microbiota fully resembles that of an adult in terms of composition and diversity [14, 15]. Whether some decline in bacterial populations and their diversity occurs in later life remains an issue of some controversy [16].

It is now abundantly evident that an intact microbiome is essential for many aspects of the development of the gastrointestinal tract including such vital components as immunological tolerance, the mucosal-, or gut-associated, immune system, epithelial and gut barrier integrity, motility, and vascularity [17–19]. That the gut microbiome can influence the development and function of more distal organs is exemplified by the emergence of the concept of the microbiota-gut-brain axis [20]. Microbiota involvement in drug metabolism may well prove to be an important contributor to the pharmacology of a number of important therapeutic agents [21].

The Disturbed Microbiome: Relevance to Human Gastrointestinal Disease

While the most compelling evidence for a role of the microbiome in the pathophysiology of a seemingly endless list of gastrointestinal and non-gastrointestinal diseases and disorders has emerged primarily from animal studies, an increasing number of observations from man support the hypotheses that have emanated from the laboratory. Before we launch into a review of these studies, it must be pointed out that most studies have been based, for obvious reasons of convenience, on the analysis of fecal samples. This approach will focus on the analysis of luminal populations and may provide little information on the bacterial population close to or adherent to the mucosal surface. These mucosal-associated bacterial species and strains will not be accurately represented in fecal samples, which is a major limitation of this approach [2, 22, 23]. It stands to reason that bacterial species resident at the mucosal surface or within the mucus layer are those most likely to participate in interactions with the host immune system, whereas those that populate the lumen may be more relevant to metabolic interactions with food or products of digestion.

Furthermore, while, in some instances, such as antibiotic-associated diarrhea, necrotizing enterocolitis, and hepatic encephalopathy, there is compelling evidence for a role for microbe-host interactions in disease pathogenesis, in others, this remains more speculative. It must be emphasized that, for most of these disorders, available data describes association and that no conclusions can be drawn with respect to causation. Let us begin with an example of the former.

Antibiotic-Associated Diarrhea

Antibiotic-associated diarrhea (AAD) provides a beautiful illustration of the delicacy of host-microbe interactions in health and the consequences of their disruption when the disease phenotype that emerges reflects complex interactions between bacterial properties, host factors, and other environmental influences. *Clostridium difficile*-associated disease (CDAD), the most feared manifestation of AAD, is a potent reminder of what can happen when we disrupt the normal microbiome, albeit with good intentions. Some individuals seem especially susceptible to the development of CDAD when administered broad-spectrum antibiotics, and it has been shown that some of this susceptibility may reside in the composition of the pre-exposure microbiota [24]. Evidence suggests that the predilection to *C. difficile*-related illness is largely a function of the resilience of the indigenous microbiota in the aftermath of an antibiotic assault, with some bacterial communities being better able to recover than others. The role of the resident microbiome in CDAD is considered a prototypical example of the emergence of a disease state when the normal, commensal microbiota and its symbiotic relationships are disturbed. Using non-sequencing methods, it has been shown that individuals who developed CDAD had a decrease in the numbers of *Bacteroides*, *Prevotella*, and *Clostridia* groups as well as higher numbers of *Enterobacteriaceae* compared with their healthy counterparts [25, 26]. Traditionally, and depending on the severity of disease, the antibiotics metronidazole and vancomycin are used as the first-line treatments in CDAD; interventions that may further impact on the normal microbiota and hence contribute to the 20 % recurrence rate that has become a worrying feature of this disease [27••]. It stands to reason, therefore, that if we are to stem the tide of a tsunami of CDAD that threatens to overrun our hospitals, we must develop new treatments for CDAD that protect the host's commensal microbiota. New generation antibiotics with microbiota-sparing properties have begun to emerge [28]. However, the role of a healthy indigenous microbiota is perhaps most dramatically illustrated by the overwhelming success of fecal microbiota transplantation (FMT) in the management of recurrent CDAD [29•]. In studies comparing patients with recurrent CDAD pre- and post-FMT, it has been shown that the intestinal microbiota changes from a low diversity state to a more diverse community with increased number of *Lachnospiraceae* and *Ruminococcaceae* following FMT [30, 31].

Inflammatory Bowel Disease

Decades of research and generations of clinical experience speak to the likely involvement of microbe-host interactions in inflammatory bowel disease (IBD). However, defining the specific and relative roles of a normal or abnormal microbiota, and the host immune response in the pathogenesis of IBD, has proven much more elusive [32••].

The fecal microbiota in Crohn's disease (CD) was found to have a distinct profile when compared with both healthy controls and ulcerative colitis (UC) patients and even when patients with ileal and colonic CD were compared [33]. The latter emphasizes the importance of phenotypic heterogeneity as a potential confounding factor in any assessment of the role of the microbiota in IBD [32••]. The finding in this study that patients with UC did not appear to have a distinct microbiome signature when compared to controls [33] contrasts with the results of a twin study [34]. Many factors may contribute to the lack of consistency between studies of the microbiota in IBD: sampling method, phenotypic heterogeneity, diet, medications, and even inflammation patterns. For example, the composition of the microbiota has been shown to differ between inflamed and non-inflamed portions of the intestine in patients with IBD [35••]; studies which fail to longitudinally assess changes in the microbiota at times of remission and relapse and in relation to symptoms and treatment will furnish data that make it difficult to distinguish between association and causality.

IBD provides a timely example of the complexity and extent of environment-microbiota-host interactions and how they can shape the microbiota and confound its interpretation. For example, it has been shown that the host's genome and the commensal microbiota interact to promote, in genetically predisposed individuals, the development of IBD in response to environmental and/or nutritional exposures during critical periods of life [35••, 36]. Of the many dietary and microenvironmental factors thought to contribute to the development of IBD, several, such as polyunsaturated fats (PUFAs) [37], short chain fatty acids (SCFAs) [38], refrigerated foods [39], dietary fiber [40], vitamin D [41], food coloring and emulsifiers [42••], prior exposure to antibiotics, [43] or helminthic infections [44, 45], have been observed to alter the microbiome and/or its interaction with the host. Epidemiological evidence supports the notion that changes in the composition of the gut microbiome during early development by any one or a combination of environmental factors may lead to persistent modifications in host immunology and physiology and to the emergence of IBD later in life [43, 46, 47].

Twin studies have provided further insights into genetic-environment interactions in IBD. Reported concordance rates for CD and UC among monozygotic twins have ranged between 27 to 56 % and 15 to 19 %, respectively [48], suggesting that genetic predisposition alone is insufficient for the development of IBD and that environmental factors play a pivotal role in disease pathogenesis, especially in UC.

Liver Disease

A considerable body of experimental and clinical evidence has emerged to implicate the gut microbiota in the development of a number of complications of chronic liver disease [49] and, more recently, in the basic pathogenesis of specific liver diseases such as alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) [50•]. In these latter instances, a combination of an altered microbiota (including its production of hepatotoxic metabolites such as alcohol and acetaldehyde), impaired gut barrier function, and a pro-inflammatory host immune response (with the release of cytokines such as tumor necrosis factor alpha (TNF-α) is thought to conspire to promote lipid deposition in the liver and incite the subsequent progression to an inflammatory liver disease [51•].

Alcohol per se disturbs the microbiota and impairs the host immune response [52]. Furthermore, its metabolites can combine with lipopolysaccharide (LPS) produced by Gram-negative bacteria to promote liver injury [53]. The microbiota contributes to alcohol-related liver injury by promoting the growth of endotoxin-producing Gram-negative bacteria in the small intestine and increasing intestinal permeability [50•]. Endotoxin, in turn, activates toll-like receptor (TLR)-4, thus activating the inflammatory cascade and driving alcohol-induced tissue injury [54]. In contrast, administration of specific probiotic organisms, such as *Lactobacillus rhamnosus*, in animal models has been shown to promote gut homeostasis by modulating the growth of Gram-negative bacteria [55] and restoring intestinal barrier integrity, reducing liver fat content and circulating levels of pro-inflammatory cytokines [56, 57]. However, with the exception of the role of antibiotics in hepatic encephalopathy, spontaneous bacterial peritonitis, and other infectious complications, little high-quality data exists on the therapeutic benefits of microbiota manipulation in human liver disease. In what may be the best such study to date, Dhiman and colleagues showed that the probiotic cocktail VSL#3 reduced the risk of hospitalization for hepatic encephalopathy, as well as Child-Turcotte-Pugh and model for end-stage liver disease scores, in patients with cirrhosis [58••].

Irritable Bowel Syndrome

Several strands of evidence suggest a potential role for a disturbed microbiota in irritable bowel syndrome (IBS) [59]. These include the occurrence de novo of IBS following an enteric infection or infestation [60], the somewhat contentious suggestion that qualitative or quantitative change in luminal bacterial populations in the small intestine, small intestinal bacterial overgrowth (SIBO), plays a major role in IBS [61, 62], and the clinical observation that some IBS sufferers respond to interventions that modify the microbiota such as antibiotics and probiotics [59]. More recently, studies of the fecal microbiota have demonstrated differences between IBS subjects and matched controls [59]. While such studies have, in general, demonstrated reduced microbial diversity in IBS [63] as well as deviations at phylum, species, and strain level [64], these findings have not been consistent between studies, perhaps due to deficiencies in study design and the intrinsic heterogeneity of any IBS population [65]. In a study that included both subjects with IBS with constipation (IBS-C) and chronic constipation, differences were demonstrated not only between their luminal and mucosal bacterial populations but also between symptoms and etiological factors such as colonic transit [66••]. It is clear that the role of the microbiota in IBS is far from settled.

Obesity and the Metabolic Syndrome

The potential role of the microbiota in the obesity epidemic has been the focus of considerable interest not just in the scientific literature but in the lay media as well. A recent review focused on the impact of the external environment on gut microbiota considering the host's geographic location and behavioral factors (diet and physical activity). These investigators also tried to delineate the relation between obesity and the microbiota [67]. Observations in animal models have indicated the ability of obese microbiota to extract more calories from the diet, thereby contributing to the development of obesity [68]. That these findings might be applicable to man are supported by the observation that transplantation of the gut microbiota collected from adult female twin pairs, discordant for obesity, into germ-free mice induced adiposity only in those mice who received the obese microbiota [69••]. Inevitably, data on the nature of gut microbiota from obese human subjects has been more variable; most studies indicate an increase in phylum *Firmicutes* and a decrease in phylum *Bacteroidetes* in association with obesity [70, 71].

Colorectal Cancer

While recent studies have identified specific signatures in gut microbiota associated with colorectal cancer (CRC) and even suggested that these may have diagnostic potential, the efficacy of this approach in clinical practice has yet to be demonstrated. In one study, a combination of the results from an analysis of the fecal microbiota together with body mass index (a known risk factor for CRC) and fecal occult blood testing provided an excellent discrimination between healthy individuals and those with malignant and premalignant lesions of the colon and rectum [72]. Other studies have linked two particular bacterial species, *Fusobacterium nucleatum* and *Escherichia coli*, with CRC [73•, 74]. While these findings are provocative, we await their application to large scale, prospective clinical trials of their diagnostic and prognostic

accuracy compared to or in conjunction with current screening modalities.

Conclusions

The advent of more rapid and less expensive sequencing and allied technologies has prompted the investigation of the gut microbiota in a number of gastrointestinal diseases and disorders and now extends to disorders beyond the gut [75]. While many changes in the composition and function of commensal bacterial populations have been described, the precise clinical significance of these findings remains unclear and, in most instances, it is premature to assign causation to apparent deviations from normal. Accessible as these technologies may be and as provocative as early observations may appear, one must continue to exert caution in the interpretation of findings from single-point-in-time analyses from small, inadequately phenotyped populations, especially when based on the analysis of fecal samples alone.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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