The Gut Microbiome, Its Metabolome, and Their Relationship to Health and Disease

Gary D. Wu
Division of Gastroenterology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract
Despite its importance in maintaining the health of the host, growing evidence suggests that gut microbiota may also be an important factor in the pathogenesis of various diseases. The composition of the microbiota can be influenced by many factors, including age, genetics, host environment, and diet. There are epidemiologic data associating diet with the development of inflammatory bowel disease as well as evidence that diet can influence both the form and the function of the microbiome. Based on this evidence, studies are now underway to examine the effect of defined formula diets, an effective therapeutic modality in Crohn’s disease, on both the gut microbiota and its metabolome as a therapeutic probe. Diet has an impact upon both the composition and the function of the microbiota in part through small-molecule production that may influence the development of both immune-mediated and metabolic diseases. By comparing dietary intake, the gut microbiota, and the plasma metabolome in omnivores versus vegans, we provide evidence that the production of certain bacterial metabolites is constrained by the composition of the gut microbiota. In total, these results demonstrate the potential promise of dietary manipulation of the gut microbiota and its metabolome as a modality to both maintain health and treat disease.

Introduction
The human gut microbiome is one of the most densely populated bacterial communities on Earth with up to $10^{11}$ organisms per gram of fecal weight composed of over a 1,000 species, most of which are obligate anaerobes [1]. The bacterial concentration, as well as complexity, increases proximally from the stomach and duodenum from approximately $10^2$ to $10^3$ aerobic organisms per gram luminal
content to $10^{11}$ to $10^{12}$ organisms per gram luminal content distally, where anaerobic organisms predominate in the cecum and colon. Throughout, the collective genome of the bacteria is 150-fold greater than that of its human host [2]. Indeed, humans should be viewed as biologic 'supraorganisms' that are dynamic and carry out functions in parallel or cooperatively. Most gut microbes are obligate anaerobes, many of which are fastidious and difficult to grow in vitro, making traditional culture techniques of limited value in characterizing the composition of the gut microbiota. The development of culture-independent methods, mainly through the use of high-throughput DNA sequencing, has provided new means to evaluate the gut microbiome and its relationship to inflammatory bowel disease (IBD). There are two primary methods that utilize deep-sequencing technologies to characterize the microbiome. The first approach uses small-subunit ribosomal RNA (16S rRNA) gene sequences (for Archaea and bacteria), or 18S rRNA gene sequences (for eukaryotes) as stable phylogenetic markers to define the lineages present in a sample [3]. Another approach uses shotgun metagenomic sequencing that permits the characterization of both the structure and the genomic representation of the microbial community. This broad-based genomic community evaluation helps to elucidate the functions encoded by the genomes of the gut microbiota. Additional technologies, such as metatranscriptomics and metaproteomics, may also provide a deeper understanding of microbial function through the direct evaluation of gene expression.

Mammalian hosts have coevolved to exist with their gut microbiota in a mutualistic relationship whereby we provide a uniquely suited environment in return for physiological benefits provided to us by our gut microbiota [4]. Examples of the latter include the fermentation of indigestible carbohydrates to produce short-chain fatty acids that are utilized by the host, biotransformation of conjugated bile acids, synthesis of certain vitamins, degradation of dietary oxalates, hydrolysis of urea by urease activity that participates in host nitrogen balance, and education of the mucosal immune system [4]. Nevertheless, there is growing evidence that the gut microbiota is associated with a number of diseases, particularly in animal model systems, but, potentially, also in humans. In this review, I will explore the relationship between diet, the bacterial gut microbiome, its metabolome, and their possible relationships to health and disease.

**Inflammatory Bowel Disease as a Paradigm for the Dysbiotic Microbiota**

The alterations in the gut microbiome that are associated with IBD are often described as being ‘dysbiotic’, or having an altered community structure, implying that there is a functional imbalance between enteric bacteria with potentially
pathogenic influences and bacteria who have a benign or beneficial effect on the host [5]. Other than the effectiveness of fecal microbiota transplantation for the treatment of recurrent Clostridium difficile infection [6], there is currently no clear evidence to confirm this notion in humans. An alternative explanation is that the observed alteration in the gut microbiome of patients with IBD is simply a consequence of the intestinal inflammatory response without consequence to the host. Growing evidence suggests that the altered composition of the dysbiotic microbiota is an adaptive response of a complex microbial community to environmental stress imposed by the intestinal inflammatory process leading to the production of electron acceptors or an increase in oxidative stress perpetuating the growth of more oxygen-tolerant organisms that belong to the Proteobacteria and Actinobacteria phyla [7, 8]. There is, however, evidence for a functional effect of a ‘dysbiotic’ intestinal microbiota in animal models [9]. Together, these studies suggest a causal role for the dysbiotic microbiota in perpetuating the chronicity of intestinal inflammation in patients with IBD.

Modulation of the Dysbiotic Microbiota for Health – The Role of Diet

Since the gut microbiota unquestionably plays a critical role in the pathogenesis of IBD, certain nongenetic factors associated with the development of IBD may be due, in part, to their effects on the gut microbiota. Environmental factors that may alter the composition of the gut microbiota include diet, the consumption of antibiotics and other xenobiotics, and geographic location. Population-based studies suggest that IBD is unevenly distributed throughout the world, with the highest disease rates occurring in industrialized nations. One theory, the hygiene hypothesis, suggests that humans living in more industrialized countries are exposed to an altered microbial environment with less complex microbial communities at an early age leading to the development of an immune system less able to ‘tolerate’ exposure to the microbial-laden environment in later life resulting in inappropriate immune activation. Consistent with this notion is the possible role of diet in light of the differences in access to clean water and availability of food refrigeration in underdeveloped parts of the world. Alternatively, a ‘westernized’ diet rich in animal fat and protein, while low in fiber, may alter the gut microbiome in a way that increases the risk for the development of IBD. Indeed, reduced richness of the gut microbiota has been shown to be associated with multiple inflammation-associated diseases and the consumption of a westernized diet relative to that found in residents of more agrarian cultures, where diets are primarily plant based [10, 11].

There are reasonable data to support a role for diet in IBD pathogenesis. Several investigators have examined the association of dietary patterns and the in-
cidence of IBD [12, 13]. For example, the authors of a systematic review concluded that high dietary intake of total fats, polyunsaturated fatty acids, ω-6 fatty acids, and meat were associated with an increased risk of Crohn’s disease and ulcerative colitis; high fiber and fruit intakes were associated with a decreased risk of Crohn’s disease, and high vegetable intake was associated with a decreased risk of ulcerative colitis [13]. These studies support a potential role for dietary patterns in the pathogenesis of IBD.

In Crohn’s disease, exclusive enteral nutrition with elemental, semi-elemental, and defined formula diets have been widely studied for the induction of remission and are considered the first-line therapy in certain parts of the world [14, 15]. These diets are also efficacious in maintaining remission [16]. Despite the efficacy of this therapeutic modality, the mechanisms by which exclusive enteral nutrition reduces inflammation in patients with Crohn’s disease are unknown. Current studies are underway to determine the effect of exclusive enteral nutrition on the composition of the gut microbiota in the hope of identifying microbial taxa and/or metabolites that are either beneficial or deleterious in Crohn’s disease pathogenesis. Conceptually, of fundamental importance to these studies is to understand how the consumption of these defined formulas are different from dietary intake of whole foods – are they providing something beneficial not delivered in whole foods or are they preventing the consumption of something deleterious in whole foods that is not present in a defined formula diet?

**Diet as a Substrate for Metabolite Production and Its Influence on Health and Disease**

Although diet can have an effect on the composition and/or richness of the gut microbiota, perhaps more important is its impact on the microbial metabolome. Indeed, diet may serve as a substrate that can be used by the gut microbiota for the production of small molecules that, after first-pass metabolism through the liver, can have an important impact on host physiology [17]. One example would be the role that gut microbiota may play in augmenting the development of atherosclerosis through the production of certain metabolites of dietary lipid phosphatidylcholine that are associated with the risk for the development of cardiovascular disease. Using a targeted approach to identify plasma metabolites which predict the cardiovascular risk in patients, Wang et al. [18] and Tang et al. [19] identified a novel pathway linking dietary lipid intake, intestinal microbiota, and atherosclerosis. Foods rich in phosphatidylcholine are a major source of choline. Catabolism of choline by the intestinal microbiota results in the formation of the gas TMA (trimethylamine) that is metabolized by the liver to form TMA oxide.
(TMAO), a small molecule that is strongly associated with the increased risk for coronary vascular disease in humans. TMAO also augments the development of atherosclerosis in animal models, thus providing the first link between dietary lipid intake, intestinal microbiota, and the risk for the development of atherosclerosis [18]. A similar pathway has been identified for the conversion of dietary carnitine, which is abundant in red meat, and its conversion into TMAO [20]. Recently, the bacterial gene family responsible for the conversion of choline into TMA, known as choline TMA-lyases, has been described [21] where investigators have shown that the greatest abundance of nonpathogenic bacterial taxa with this gene representation are located in three of the four major phyla of the human gut microbiome. With this knowledge, several possible avenues can be envisioned by which this information can now be used to develop technologies that may directly impact upon human health.

Another example, which is perhaps more relevant to the pathogenesis and, possibly, the treatment of immune-mediated diseases such as IBD, would be the delivery of indigestible carbohydrates to the gut microbiota through dietary intake leading to the production of short-chain fatty acids by bacterial fermentation that play a role in immune function [22, 23], intestinal hormone production, and lipogenesis [24]. Exploiting this relationship between diet and the gut microbiota is a strategy to treat immune-mediated diseases by restoring immune tolerance by the activation of regulatory T cells. For example, additional prebiotics could deliver fermentable substrates to an enhanced microbiota fortified by ‘next-generation’ probiotics designed to alter the composition of the gut microbiota to produce greater levels of short-chain fatty acids that would, in turn, activate specific G-protein-coupled receptors to decrease inflammation. An alternative approach would be to develop a small molecule to directly target the activation of specific G-protein-coupled receptors known to modulate immune function [22].

**Impact of Diet on the Composition of the Human Microbiota and Its Production of Metabolites**

Prior studies in animal models and globally distinct human populations focusing on a ‘westernized’ versus an agrarian plant-based diet suggest that the impact of diet on taxonomy may be large, implying that the gut microbiota plays a significant role in health [25–27]. By contrast, more moderate dietary interventions, which can be sustained in humans on the long term, suggest that the impact of diet may be more modest [28]. To examine the impact of a plant-based diet within the context of a westernized environment, we compared measures of
dietary intake, gut microbiota composition, and the plasma, urinary, and fecal metabolome of healthy human subjects consuming either a long-term vegan or omnivorous diet [29]. Vegans consumed significantly less micronutrients associated with protein and fat intake than omnivores, whereas carbohydrate consumption was significantly greater. The plasma metabolome of vegans differed markedly from that of omnivores, but the gut microbiota was surprisingly similar. Unlike prior studies of individuals living in agrarian societies [26], the higher consumption of fermentable substrate in vegans was not associated with higher levels of fecal short-chain fatty acids, a finding confirmed in a 10-day controlled feeding experiment [28]. Similarly, the proportion of vegans capable of producing equol, a soy-based gut microbiota metabolite, was less than reported in Asian societies despite the high consumption of soy-based products. The notion that differences in the composition of the gut microbiota in globally distinct cultures might impact upon function is also supported by the identification of a specific gut bacterial species capable of seaweed algal metabolism found in the gut microbiota of the Japanese but not in residents of North America [30]. These results suggest that environmental factors that shape the composition of the gut microbiota may have a significant effect on the relationship between substrates provided to the gut microbiota through dietary consumption of the host and the production of microbial metabolites.

**Conclusion**

There is growing interest in targeting the gut microbiota as a strategy to maintain health and treat disease. Advances in DNA sequencing and mass spectrometry technologies together with enhanced biocomputational tools designed to analyze high-dimensional data sets have identified microbial taxa and metabolites that could be developed as therapeutic strategies to target specific disease states through the development of next-generation pre-, pro-, and synbiotics. The effect of diet on both the composition of the gut microbiota and its production of metabolites is likely to be a very important component of this strategy. Additional studies are needed to characterize environmental factors independent of diet which may play a critical role in shaping the composition of the gut microbiota in globally distinct human societies which, in turn, may have an effect on the production of beneficial metabolites, such as short-chain fatty acids, from the diet and gut microbiota. The development of prebiotics to deliver substrates for the gut microbiota to produce desirable metabolites that will favor health may need to take differences in the composition of the gut microbiota in various societies around the world into consideration.
Disclosure Statement

The author has received research funding and honoraria from Nestle for organizing and/or speaking in scientific symposia.

References