Manipulating the Gut Microbiome as a Therapy for IBD

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Abstract
The human gut contains a vast number of microorganisms known collectively as the gut microbiota. Despite its importance in maintaining the health of the host, growing evidence suggests the gut microbiota may also be an important factor in the pathogenesis of various diseases, a number of which have shown a rapid increase in incidence over the past few decades. In some of these diseases, such as inflammatory bowel disease (IBD), the microbiota is dysbiotic with an abnormal community structure and decrease in diversity. If the dysbiotic microbiota plays a role in disease pathogenesis, interventions that modify its composition to make it more similar to the microbiota observed in health, might be a strategy to treat certain disease processes. Indeed, the high-level efficacy of fecal microbiota transplantation in the treatment of refractory Clostridia difficile infection supports this notion as proof-of-principle. The composition of the microbiota can be influenced by many factors including age, genetics, host environment, and diet. With respect to the later, diet has an impact upon both the composition and function of the microbiota. There are epidemiologic data associating diet with the development of IBD as well as evidence that diet can influence both the form and function of the microbiome in a manner that impacts upon the development of intestinal inflammation. 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 microbiome and its metabolome as a therapeutic probe with the hope of better defining the ‘healthy’ diet in patients with IBD.

Diet, the Gut Microbiome and the Metabolome in IBD

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Abstract
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**Introduction**

Human microbiomes are very distinctive amongst various body sites and are composed of not only bacteria but also other microorganisms including other prokaryotic organisms such as Archaea, microeukaryotes such as fungi, and viruses, principally bacteriophage – the latter being amongst the most abundant biologic entities in the biosphere. In this review, the term microbiota will be used to denote the compilation of bacterial microorganisms within a specific environment, whereas the microbiome refers to not only the bacterial taxa but also their collective genomes. The human gut microbiota is a densely populated bacterial community with approximately $10^{11}$ organisms per gram of fecal weight composed of more than 1,000 species, most of which are obligate anerobes [1, 2], with a collective genome size 150-fold greater than that of its human host [1]. Although there are over 50 bacterial phyla on Earth, human-associated bacteria largely belong to one of four phyla, Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes. Mammalian hosts have coevolved to exist with our gut microbiota in a mutualistic relationship, where we provide a uniquely suited environment in return for physiological benefits provided to us by our gut microbiota [3]. 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, the hydrolysis of urea by urease activity that participates in host nitrogen balance, and education of the mucosal immune system [3].

**The Association between the Gut Microbiota and Human Disease**

Despite the importance of the gut microbiota in maintaining the health of the host, growing evidence suggests that it may also be an important factor in the pathogenesis of a variety of diseases, particularly those that have shown a rapid increase in incidence over the past few decades. These include both type 1 and type 2 diabetes mellitus, atherosclerosis, asthma, colon cancer, and inflammatory bowel disease (IBD), to name a few [4]. Advanced genomic technology, principally DNA sequencing and SNP mapping used for genome-wide association studies combined with biocomputational algorithms, have revealed the genetic underpinnings of these complex disease processes. In most circumstances, the contribution of host genetics to the risk of disease development is significantly less than 50%, implicating the importance of environmental influences [5]. The observation that these diseases have shown a steadily increasing incidence over the past several decades, the geographic distribution of disease clus-
tering in industrialized nations, and immigration studies revealing the adoption of disease risk of the host country within one or two generations all emphasize further the importance of environment in the pathogenesis of these diseases.

Interestingly, inflammation has been strongly associated with many of these ‘Westernized’ disease processes. In addition to IBD and asthma, which are principally diseases due to unrestrained immune processes, T1DM has been associated with type 1 interferon production and altered T cell signaling, suggesting an autoimmune response and insulin resistance, the hallmark of T2DM, and is associated with an inflammatory response in adipose tissue [6]. Even obesity and atherosclerosis have been associated with chronic inflammation with elevations of serologic markers such as C-reactive protein.

Although a causal relationship has been demonstrated primarily in animal models and a functional effect in human disease is currently lacking, the role that the gut microbiota plays in the establishment of host immunity together with its effects on the inflammatory response suggests that continued investigation may lead to direct evidence for the role of the microbiota in at least some of these disease processes. Indeed, early-life exposure to the gut microbiota and its effects on the development of immunologically based disease have recently been demonstrated in an animal model [7].

**Determinants of Gut Microbiota Composition, Dysbiosis and IBD**

Using our current understanding of disease pathogenesis in IBD as a paradigm, functional genomics has revealed a complex interaction between host innate and adaptive immunity that provides protection against microbial invasion, yet demonstrates tolerance to colonization with the microbiota at mucosal surfaces (recently reviewed in Khor et al. [8]). In the case of IBD, the loss of mucosal tolerance, together with a defect in protective host innate immunity to a dysbiotic microbiota, leads to an unrestrained mucosal immune response, the hallmark of this disease process. Indeed, of all the chronic disease states currently associated with the gut microbiota, the evidence for a causative role in the pathogenesis of IBD is the strongest.

Dysbiosis of the gut microbiota, an alteration of the microbial community structure associated with disease, has been consistently observed in patients with IBD. Although the dysbiosis may simply be a result of the inflammatory process [9], it may play a role in the pathogenesis of disease, where there is an increase in potentially harmful and a reduction in more protective bacterial species [10]. Functional evidence for a role of the dysbiotic microbiota in the pathogenesis of disease is supported by animal models, where colitis, or predisposition to dis-
ease, can be transferred to wild-type mice using genetically defined disease mice as gut microbiota donors [11, 12].

The notion of an alteration in the composition of the gut microbiota as a possible etiologic factor in the predisposition to immunologically mediated disease has been proposed as one of the environmental factors that may play in a role in the increasing incidence of the diseases associated with the gut microbiota mentioned previously [13]. Indeed, many aspects of our environment have been changed dramatically over the past few decades concurrent with the increasing incidence of these disease processes. Elements of the modern lifestyle that have been postulated to result in changes in the gut microbiota include improved sanitation, vaccinations, increased antibiotic use, decline in parasite infections, caesarean section, decline in Helicobacter pylori, smaller family size, refrigeration, less crowded living conditions, sedentary lifestyles, food processing, and dietary changes. The impact of host genetics on the gut microbiota may also play a role, but evidence is largely based on studies in model organisms such as rodents and has been reviewed recently [14]. The impact of host genetics on the gut microbiota in healthy human populations, based on current evidence [15], may be relatively modest, but further studies are needed.

**Short- versus Long-Term Diet and the Gut Microbiota**

The impact of diet on the composition of the gut microbiota begins early in life. Colonization of the gut begins at birth and, following an initial chaotic community structure during the first year of life, the human gut microbiota becomes more stable and adult-like [16] concurrent with the introduction of solid foods into the diet [17]. However, it is not until 2–3 years of age that the gut microbiota in childhood strongly resembles that of the adult [18]. Several studies have explored the impact of diet on the newborn gut microbiota and have compared breastfeeding with formula feeding. A consistent finding has been the higher proportion of Bifidobacteria in breastfed infants as compared to formula-fed infants [19–22].

Although studies have examined the impact of diet on the gut microbiome of various mammalian species in a cross-sectional fashion [23] as well as in a mouse intervention study [24], until very recently, there have not been any studies broadly examining the association between diet and the composition of the adult human gut microbiota. We recently reported two separate experiments, whereby the association between diet and the human gut microbiota was evaluated in a cross-sectional and short-term interventional study [25]. Dietary questionnaires, used to assess dietary consumption and 16S rRNA gene sequencing to
Determine the composition of the gut microbiota in 98 healthy human subjects, revealed a statistically significant association between overall diet and the composition of the gut microbiota. Spearman correlation coefficient associations between bacterial taxa and micronutrients revealed that major nutrient categories clustered independently with relative proportions of bacterial taxa, where ‘fat’ and ‘fiber’ were inversely correlated as were ‘amino acids’ and ‘carbohydrates’ [26]. These observations are also consistent with a study comparing the gut microbiota of children from a village in the West African country of Burkina Faso with those in Europe [26], as well as a more recent study comparing the gut microbiome of residents in the agrarian Malawi and Amerindian societies with residents in the US [18], where the inverse relationship between Bacteroides and Prevotella genera were also noted. Although the abundance of Prevotella could be considered a discriminative taxon associated with the residence within an agrarian society, the fact that these associations were also observed in residents of the US [25] supports the notion that the observed inversely related proportions of Prevotella and Bacteroides may be, in part, due to diet.

A second study to evaluate the impact of a short-term dietary intervention with either a low fat/high fiber or high fat/low fiber on the human gut microbiota revealed that the gut microbiota responds to a dietary change within 24 h [25]. Although the short-term diet-induced alteration in the gut microbiota was highly statistically significant, the effect was modest relative to inherent intersubject variability in gut microbiota composition. Furthermore, we did not find any evidence that short-term diet reduced this intersubject variability. In addition, broad taxonomic alterations, often observed in mice to be associated alterations in fat/fiber [27, 28] were not observed in this short-term dietary intervention in humans, supporting the notion that long-term dietary alterations may be needed to broadly alter the composition and/or richness (number of distinct bacterial taxa) of the gut microbiota in humans [25, 29, 30]. Clearly, further investigation into this important topic is needed.

**Diet and the Structure versus Function of the Gut Microbiome: Impact on the Metabolome and the Identification of Therapeutic Targets**

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 [31]. An example of this would be the delivery of indigestible carbohydrates to the gut microbiota through
dietary intake leading to the production by bacterial fermentation of short-chain fatty acids that play a role in immune function [32, 33], intestinal hormone production and lipogenesis [34]. Another 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 (CVD). Using a targeted approach to identify plasma metabolites which predict CVD in patients, Wang et al. [35] and Tang et al. [36] 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, the intestinal microbiota, and the risk for the development of atherosclerosis [35]. A similar pathway has been identified for conversion of dietary carnitine, which is high in red meat, and its conversion into TMAO [25, 37].

Recently, the bacterial gene family responsible for the conversion of choline into TMA, known as choline TMA-lyases, has been described [38], where investigators have shown that the greatest abundance of nonpathogenic bacterial taxa with this gene representation is 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: (1) quantify the risk for heart disease attributed to the consumption of choline by characterizing the abundance of bacteria in the gut that have a choline TMA-lyase gene; (2) design an approach to reduce or extinguish TMA-lyase-expressing bacteria in the gut; (3) develop drugs to inhibit TMA-lyase activity in bacteria, and (4) develop ‘designer foods’ or ‘medical foods’ to reduce the production of TMA by bacteria from the diet. These concepts are of considerable importance to the field as scientists search for opportunities, whereby the knowledge gained from our understanding of the gut microbiome can be used to prevent and/or treat human disease.

**Diet, the Gut Microbiota and 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 use of antibiotics,
and geographic location. Population-based studies suggest that IBD is unevenly distributed throughout the world with the highest disease rates occurring in industrialized nations [39, 40]. One theory, the hygiene hypothesis, suggests that humans living in more industrialized countries are exposed to fewer microbes or 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.

Regardless of the mechanism, there are reasonable data to support a role for diet in IBD pathogenesis. Several investigators have examined the association of dietary patterns and the incidence of IBD [41, 42]. For example, the authors of a systematic review concluded that high dietary intake of total fats, polyunsaturated fatty acids, omega-6 fatty acids, and meat were associated with an increased risk of CD and UC; high fiber and fruit intakes were associated with a decreased CD risk, and high vegetable intake was associated with a decreased UC risk [42]. These studies support a potential role for dietary patterns in the pathogenesis of IBD. As proof of principle, the consumption of milk fat has been shown to alter host bile acid composition, thereby promoting the expansion of the sulphite-reducing pathobiont *Bilophila wadsworthia*, resulting in an exacerbation of colitis in IL-10 KO mice [43]. Together with the recent data characterizing the impact of diet on the gut microbiome [25], it is tempting to speculate that the alteration of gut microbiota community structure through the consumption of agrarian versus a Westernized diet may play a role in either reducing or increasing, respectively, the risk for the development of IBD.

In Crohn’s disease, exclusive enteral nutrition (EEN) with elemental, semi-elemental, and defined formula diets has been widely studied for induction of remission and is considered first-line therapy in certain parts of the world [44, 45]. These diets are also efficacious in maintaining remission [46]. The most common protocol involves the administration of a defined formula at 100% of caloric needs for 4–12 weeks in order to induce remission [47]. A smaller percentage of calories provided by the defined formula may be required in order to maintain remission, allowing additional flexibility in the diet [46]. Despite the efficacy of this therapeutic modality, the mechanisms by which EEN reduces inflammation in patients with Crohn’s disease are unknown. Current studies are underway to determine the effect of EEN 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 is different from dietary intake of whole foods.

Conclusions

In this review, we highlight diseases for which the gut microbiota has been implicated in disease pathogenesis, focusing on those associated with the consumption of a Westernized diet. Although studies now suggest that diet has an impact on the human gut microbiota, there is clearly much to be learned. The impact of diet on the composition of the microbiota may ultimately be less important to host physiology than its impact on the microbial metabolome, where the production of a multitude of small molecules may have important consequences for human health and disease [30]. Additionally, associations between the gut microbiota and human disease, including the impact of diet, do not prove cause-and-effect relationships. Indeed, most data supporting a functional effect of an altered microbiota on host physiology are based primarily on murine models. Although such studies provide fundamentally important information about disease mechanisms demonstrating proof of principle, the degree to which they reflect human pathophysiology awaits further investigation.

Disclosure Statement

The author declares that no financial or other conflict of interest exists in relation to the content of the chapter.

References


