Gut Bacteria in Health and Disease

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Abstract: A new era in medical science has dawned with the realization of the critical role of the “forgotten organ,” the gut microbiota, in health and disease. Central to this beneficial interaction between the microbiota and host is the manner in which bacteria and most likely other microorganisms contained within the gut communicate with the host’s immune system and participate in a variety of metabolic processes of mutual benefit to the host and the microbe. The advent of high-throughput methodologies and the elaboration of sophisticated analytic systems have facilitated the detailed description of the composition of the microbial constituents of the human gut, as never before, and are now enabling comparisons to be made between health and various disease states. Although the latter approach is still in its infancy, some important insights have already been gained about how the microbiota might influence a number of disease processes both within and distant from the gut. These discoveries also lay the groundwork for the development of therapeutic strategies that might modify the microbiota (eg, through the use of probiotics). Although this area holds much promise, more high-quality trials of probiotics, prebiotics, and other microbiota-modifying approaches in digestive disorders are needed, as well as laboratory investigations of their mechanisms of action.

Due largely to rapidly evolving advances in analytic techniques in microbiology, molecular biology, and bioinformatics, the true diversity of microorganisms that inhabit the gastrointestinal tract of humans (collectively referred to as the human gut microbiota) is being revealed and its contributions to homeostasis in health and to the pathogenesis of disease appreciated (Table 1). As a consequence, the study of gut ecology has emerged as one of the most active and exciting fields in biology and medicine. It is in this context that maneuvers to alter or modify the microbiota, either through dietary modifications or by the administration of antibiotics, probiotics, or prebiotics, must now be viewed.
The Normal Gut Microbiota: An Essential Factor in Health

Basic Definitions and Development of the Microbiota

The term microbiota is to be preferred to the older term flora, as the latter fails to account for the many nonbacterial elements (eg, archaea, viruses, and fungi) that are now known to be normal inhabitants of the gut. Given the relatively greater understanding that currently exists of the role of bacteria, in comparison with the other constituents of the microbiota in health and disease, gut bacteria will be the primary focus of this review. Within the human gastrointestinal microbiota exists a complex ecosystem of approximately 2 million genes (the microbiome). Indeed, the number of bacteria within the gut is approximately 10 times that of all the cells in the human body, and the collective bacterial genome is vastly greater than the human genome.

At birth, the entire intestinal tract is sterile; the infant’s gut is first colonized by maternal and environmental bacteria during birth and continues to be populated through feeding and other contacts. Factors known to influence colonization include gestational age, mode of delivery (vaginal birth vs assisted delivery), diet (breast milk vs formula), level of sanitation, and exposure to antibiotics. The intestinal microbiota of newborns is characterized by low diversity and a relative dominance of gram-negative organisms and anaerobes. Thereafter, the microbiota becomes more diverse with the emergence of the dominance of Firmicutes and Bacteroidetes; this period of maturation of the microbiota may be critical; there is accumulating evidence from a number of sources that disruption of the microbiota in early infancy may be a critical determinant of disease expression in later life. It follows that interventions directed at the microbiota later in life may, quite literally, be too late and potentially doomed to failure.

Following infancy, the composition of the intestinal microflora remains relatively constant until later life. Although it has been claimed that the composition of each individual’s flora is so distinctive that it could be used as an alternative to fingerprinting, more recently, 3 different enterotypes have been described in the adult human microbiome. These distinct enterotypes are dominated by Prevotella, Ruminococcus, and Bacteroides, respectively, and their appearance seems to be independent of sex, age, nationality, and body mass index. The microbiota is thought to remain stable until old age when changes are seen, possibly related to alterations in digestive physiology and diet. Indeed, Claesson and colleagues were able to identify clear correlations in elderly individuals, not only between the composition of the gut microbiota and diet, but also in relation to health status.

Regulation of the Microbiota

Because of the normal motility of the intestine (peristalsis and the migrating motor complex) and the antimicrobial effects of gastric acid, bile, and pancreatic and intestinal secretions, the stomach and proximal small intestine, although certainly not sterile, contain relatively small numbers of bacteria in healthy subjects. Interestingly, commensal organisms with probiotic properties have recently been isolated from the human stomach. The microbiology of the terminal ileum represents a transition zone between the jejunum, containing predominantly aerobic species, and the dense population of anaerobes found in the colon. Bacterial colony counts may be as high as 10^9 colony-forming units (CFU)/mL in the terminal ileum immediately proximal to the ileocecal valve, with a predominance of gram-negative organisms and anaerobes. On crossing into the colon, the bacterial concentration and variety of the enteric flora change dramatically. Concentrations of 10^{12} CFU/mL or greater may be found and are comprised mainly of anaerobes such as Bacteroides, Porphyromonas, Bifidobacterium, Lactobacillus, and Clostridium, with anaerobic bacteria outnumbering aerobic bacteria by a factor of 100 to 10000:1. The predominance of anaerobes in the colon reflects the fact that oxygen concentrations in the colon are very low; the flora has simply adapted to survive in this hostile environment.

At any given level of the gut, the composition of the flora also demonstrates variation along its diameter, with certain bacteria tending to be adherent to the mucosal surface, while others predominate in the lumen. It stands...
Host-Microbiota Interactions

Gut-commensal microbiota interactions play a fundamental role in promoting homeostatic functions such as immunomodulation, upregulation of cytoprotective genes, prevention and regulation of apoptosis, and maintenance of barrier function. The critical role of the microbiota on the development of gut function is amply demonstrated by the fate of the germ-free animal. Not only are virtually all components of the gut-associated and systemic immune systems affected in these animals, but the development of the epithelium, vasculature, neuromuscular apparatus, and gut endocrine system also is impaired. The subtleties of the interactions between the microbiota and the host are exemplified by studies that demonstrate the ability of a polysaccharide elaborated by the bacterium Bacteroides fragilis to correct T-cell deficiencies and Th1/Th2 imbalances and direct the development of lymphoid organs in the germ-free animal. Intestinal dendritic cells appear to play a central role in these critical immunologic interactions.

How does the gut immune system differentiate between friend and foe when it comes to the bacteria it encounters? At the epithelial level, for example, a number of factors may allow the epithelium to tolerate commensals (and thus probiotic) organisms. These include the masking or modification of microbial-associated molecular patterns that are usually recognized by pattern recognition receptors, such as Toll-like receptors, and the inhibition of the NFκB inflammatory pathway. Responses to commensals and pathogens also may be distinctly different within the mucosal and systemic immune systems. For example, commensals such as Bifidobacterium infantis and Faecalibacterium prausnitzii have been shown to differentially induce regulatory T cells and result in the production of the anti-inflammatory cytokine interleukin (IL)-10. Other commensals may promote the development of T-helper cells, including Th17 cells, and result in a controlled inflammatory response that is protective against pathogens in part, at least, through the production of IL-17.

Through these and other mechanisms, the microbiota can be seen to play a critical role in protecting the host from colonization by pathogenic species. Some intestinal bacteria produce a variety of substances, ranging from relatively nonspecific fatty acids and peroxides to highly specific bacteriocins, which can inhibit or kill other potentially pathogenic bacteria, while certain strains produce proteases capable of denaturing bacterial toxins.

The Microbiota and Metabolism

Although the immunologic interactions between the microbiota and the host have been studied in great detail for some time, it has been only recently that the true extent of the metabolic potential of the microbiota has begun to be grasped. Some of these metabolic functions were well known, such as the ability of bacterial disaccharidases to salvage unabsorbed dietary sugars, such as lactose, and alcohols and convert them into short-chain
fatty acids (SCFAs) that are then used as an energy source by the colonic mucosa. SCFAs promote the growth of intestinal epithelial cells and control their proliferation and differentiation. It has also been known for some time that enteric bacteria can produce nutrients and vitamins, such as folate and vitamin K, deconjugate bile salts, and metabolize some medications (such as sulfasalazine) within the intestinal lumen, thereby releasing their active moieties. However, it is only recently that the full metabolic potential of the microbiome has come to be recognized and the potential contributions of the microbiota to the metabolic status of the host in health and in relation to obesity and related disorders have been appreciated. The application of genomics, metabolomics, and transcriptomics can now reveal, in immense detail, the metabolic potential of a given organism. 

It is now also known that certain commensal organisms also produce other chemicals, including neurotransmitters and neuromodulators, which can modify other gut functions, such as motility or sensation. Most recently and perhaps most surprisingly, it has been proposed that the microbiota can influence the development and function of the central nervous system, thereby leading to the concept of the microbiota-gut-brain axis.

The Gut Microbiota in Disease

Just as we are only now beginning to understand the key role of the flora in health, it has only been in very recent years that the true extent of the consequences of disturbances in the flora, or in the interaction between the flora and the host, has been recognized. Some of these consequences are relatively obvious. For example, when many components of the normal flora are eliminated or suppressed by a course of broad-spectrum antibiotics, the stage is set for other organisms that may be pathogenic to step in and cause disease. The classic example of this is antibiotic-associated diarrhea and its deadliest manifestation, Clostridium difficile colitis. Similar perturbations in the flora are thought to be involved in a devastating form of intestinal inflammation that may occur in newborns and especially premature infants: necrotizing enterocolitis. In other situations, bacteria may simply be where they should not be. If motility of the bowel is impaired and/or acid secretion from the stomach is drastically reduced, an environment conducive to the proliferation of organisms in the small intestine that are normally confined to the colon results; the consequence is the syndrome of small bowel bacterial overgrowth. In other situations, the immunologic interaction between the flora and the host is disturbed, and the host may, for example, begin to recognize the constituents of the normal flora not as friend but as foe and may mount an inappropriate inflammatory response, which, some believe, may ultimately lead to conditions such as IBD.

Most recently, qualitative changes in the microbiota have been invoked in the pathogenesis of a global epidemic: obesity. It has been postulated that a shift in the composition of the flora toward a population dominated by bacteria that are more avid extractors of absorbable nutrients—which are then available for assimilation by the host—could play a major role in obesity. Such studies rely on the application of modern technologies (genomics, metagenomics, and metabolomics) to the study of the colonic flora and have the potential to expose the true diversity and metabolic profile of the microbiota and the real extent of changes in disease. Rather than provide an exhaustive survey of all the disease states that might be influenced by the microbiota, a brief overview of current information on the role of the microbiota in a few common diseases/disorders will be provided below.

Inflammatory Bowel Disease

There is a considerable body of evidence to support the hypothesis that the endogenous intestinal microflora plays a crucial role in the pathogenesis of IBD and its variants and related disorders. Some of this evidence is time-honored, such as the predilection of IBD for areas of high bacterial numbers and the role of contact with the fecal stream in sustaining inflammation. Other evidence is more recent and includes studies described above that illustrate the key roles of the microbiota in host immune responses and the generation of inflammatory responses. This evidence is supplemented by experimental observations on the ability of strategies that modify the microbiota (eg, the administration of probiotics) to modulate the inflammatory response in experimental models of IBD. Studies of the gut microbiota in IBD have revealed quantitative and qualitative changes, including the intriguing finding in some studies that a bacterium with anti-inflammatory properties, F prausnitzii, is less abundant in patients with IBD than in healthy individuals. The importance of microbiota-host interactions in IBD is further supported by the many studies of IBD genetics that have identified a host of changes in genes that code for molecules involved in bacterial recognition, host-bacteria engagement, and the resultant inflammatory cascade. On a more clinical level, the role of the microbiota is supported by the efficacy, albeit variable, of antibiotics in IBD and the suggestion,
Irritable Bowel Syndrome
A variety of strands of evidence suggest a role for the gut microbiota in IBS (Table 2). First and foremost among these is the clinical observation that IBS can develop in individuals de novo following exposure to enteric infections and infestations (ie, postinfectious IBS). More contentious has been the suggestion that patients with IBS may harbor small intestinal bacterial overgrowth (SIBO). More indirect evidence of a role for the microbiota can be gleaned from some of the metabolic functions of the components of the microbiota. Thus, given the effects of bile salts on colonic secretion, changes in bile salt deconjugation could lead to changes in stool volume and consistency. Similarly, changes in bacterial fermentation could result in alterations in gas volume and/or composition. Further evidence comes from the clinical impact of therapeutic interventions, such as antibiotics, prebiotics, or probiotics, which can alter or modify the microbiota. Thus, the poorly absorbed antibiotic rifaximin (Xifaxan, Salix) has been shown to alleviate symptoms in diarrhea-predominant IBS, and some probiotics (B infantis 35624 [Align, Procter & Gamble] in particular) have been shown to exert substantial clinical responses. The latter is of interest, given its demonstrated ability to modulate the systemic immune response in humans. Also gaining currency is the suggestion that the colonic microbiota may demonstrate qualitative and/or quantitative changes in IBS.

Modern molecular microbiologic methods are now being applied to this complex issue and have, indeed, confirmed that patients with IBS, regardless of subtype, do exhibit a fecal flora that is clearly different from that of control subjects. Studies by my colleagues and I have demonstrated, firstly, a reduced microbial diversity in IBS and, secondly, using high-throughput pyrosequencing, the existence of different IBS subgroups based on a detailed examination of the microbiota. At the phylum level, 1 of these subgroups resembled control subjects, whereas another demonstrated a shift in the relative proportion of the 2 major phyla, Firmicutes and Bacteroidetes, as well as significant changes at species and strain levels. The primacy of these microbial shifts and their potential to disturb...
mucosal or myoneural function in the gut wall, impact the brain-gut axis, or induce local or systemic immune responses remains to be defined (Figure 1). Most intriguing has been the suggestion, from animal studies, that the gut microbiota can influence brain function and morphology.49 Several experimental observations provide a scientific basis for the use of therapies that might modify the microbiota in IBS.85-87 Thus, oral administration of B infantis 35624 has been shown to attenuate interferon γ, tumor necrosis factor α (TNF-α), and IL-6 responses following mitogen stimulation, increase plasma levels of tryptophan and kynurenic acid, and, most strikingly, reduce concentrations of 5-hydroxyindoleacetic acid and dihydroxyphenylacetic acid concentrations in the frontal cortex and amygdala, respectively.88

These observations were taken one step further by the same research group by demonstrating normalization of immune responses, reversal of behavioral deficits, and restoration of basal norepinephrine concentrations in the brainstem in an animal model of depression (the maternally separated rat).89 While these latter observations could address some of the proposed pathophysiologic mechanisms associated with symptom development in IBS, namely, immune activation and disturbances in the brain-gut axis, other studies suggest that the same strain can also modify peripheral mechanisms linked with IBS, such as visceral hypersensitivity.90

Addressing another gut abnormality identified in IBS, Zeng and colleagues partially reversed changes in small intestinal permeability with a probiotic cocktail.91 Another organism, Lactobacillus acidophilus, has been shown to produce visceral analgesic effects through the induction of μ-opioid and cannabinoid receptors,92 and Lactobacillus paracasei has been shown to attenuate gut muscle hypercontractility in an animal model of postinfectious IBS.93 Again, this effect was strain-dependent and appeared to be mediated, in part, through a modulation of the immunologic response to the initial infection and, in part, through the direct effects of the organism, or a metabolite thereof, on gut muscle. In other experiments, this same organism was capable of attenuating antibiotic-induced visceral hypersensitivity in mice.94

Figure 2. The gut flora (microbiota) and the liver. Small intestinal bacterial overgrowth (SIBO), present in a variety of liver diseases, and/or an altered composition of the colonic microbiota lead to an enhanced release of proinflammatory cytokines. Increased intestinal permeability, also well described in liver disease, enhances translocation of bacteria, endotoxin, or proinflammatory products such as lipopolysaccharide (from gram-negative bacteria), which reach the liver through the portal vein or, in the presence of portal-systemic shunting, access the systemic circulation directly.
Lactobacillus reuteri also has been shown to inhibit visceral pain induced by colorectal distension in the rat. Of clinical relevance, this same probiotic organism has been shown to readily colonize and induce an immune response in the small intestine in humans. Interestingly, in view of the relevance of tachyphylactic acid biosynthesis in the immunologic responses to certain lactobacilli, it has been shown by Duncker and colleagues that a Lactobacillus mutans (leading to D-alanine depletion of lipoteichoic acid) also significantly inhibited visceral pain perception in healthy noninflamed rats.

Functional and morphologic changes in the enteric neuromuscular apparatus develop in mice infected with Trichinella spiralis long after the worms have been expelled and the related inflammatory response has subsided, thus providing an animal model of postinfectious IBS. L. paracasei, but not other strains, has been shown to attenuate gut muscle hypercontractility, reduce immune activation, and normalize the metabolic profile of mice in this model.

These experimental observations are now supported by clinical studies with probiotics in IBS in humans. Results in IBS continue to be variable with a number of organisms, such as Lactobacillus GG, Lactobacillus plantarum, L. acidophilus, Lactobacillus casei, the probiotic cocktail VSL#3, and Bifidobacterium animalis, alleviating individual IBS symptoms (eg, bloating, flatulence, and constipation) and only a few products affecting pain and global symptoms. Other products have shown no benefit.

Obesity, Metabolic Syndrome, Nonalcoholic Fatty Liver Disease, and Nonalcoholic Steatohepatitis

Several mechanisms involving the microbiota in the pathogenesis of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) have been identified. In particular, a role for the microbiota in relation to diet in the pathogenesis of obesity per se has been extensively investigated. Pertinent findings include the ability of gram-negative anaerobes, such as Bacteroides thetaiotaomicron, to cleave most glycosidic linkages and degrade plant oligosaccharides, thereby supplying the host with 10% to 15% of its calorific requirement. The microbiota of obese individuals, as well as the cecal microbiota of ob/ob mice, is more efficient at the extraction of energy from the diet and in the production of SCFAs. Furthermore, the microbiota has been shown to stimulate hepatic triglyceride production through suppression of the lipoprotein lipase (LPL) inhibitor, fasting-induced adipose factor (also known as angiopoietin-like 4), thereby leading to continued expression of LPL, a key regulator of fatty acid release from triglycerides in the liver. The gut microbiota also can modulate systemic lipid metabolism through modification of bile acid metabolic patterns, also impacting directly on the emulsification and absorption properties of bile acids and, thus, indirectly on the storage of fatty acids in the liver. The microbiota also has been implicated in the development of insulin resistance, a fundamental abnormality in metabolic syndrome, by affecting energy balance, glucose metabolism, and the low-grade inflammatory state that has been associated with obesity and related metabolic disorders. Its role in choline metabolism, as well as inactivation of proinflammatory cytokines (eg, TNF-α), appears relevant to the development of NAFLD and progression to NASH. Most recently, studies in experimental models have shown that defective/deficient inflammasome sensing and related dysbiosis result in an abnormal accumulation of bacterial products in the portal circulation and promote progression of NAFLD/NASH.

A more fundamental role for SIBO has been proposed in NAFLD by promoting both steatosis and inflammation (Figure 2). The potential of microbes of enteric origin to induce a progressive and even fatal steatohepatitis had been recognized several years ago in relation to the liver injury that complicated jejun-ileal bypass operations for morbid obesity; indeed, that procedure has provided a valuable experimental model for exploring the impact of the microbiota in liver disease.

Summary

The true diversity and function of the human gut microbiota as well as the extent and nature of its interactions with the host continue to be revealed; although much progress has been made in a very short time, the story is by no means complete, and the impact of a number of host, bacterial, and environmental factors on the composition and function of the microbiota is just beginning to be recognized. These factors must be taken into account when interpreting changes in the microbiota reported in disease states, and caution should be exercised in attributing a causative role to microbial changes seen in any disease state until much more is known of the primacy of these changes in that disorder. Some of the observed deviations in microbiota composition observed in a disease may be no more than epiphenomena. Nevertheless, as the critical role of the heretofore “ignored organ”—the gut microbiota—in health and disease has come to be recognized, so has the possibility that modifying the flora might be of therapeutic benefit.

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animal disorders and holds patents in these areas. He is also a consultant to Almirall, Forest, Ironwood, Rhythm, Salix, Shire-Movisit, and Tioga and has received honoraria for speaking engagements from Danone, Korea Yakult, Proctor & Gamble, and Yakult and research support from GlaxoSmithKline, Norgine, and Proctor & Gamble.

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