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The Gut Microbiome – Clinical Implications for the Practicing Gastroenterologist

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The Human Gut Microbiome: The Basics

In recent years biomedical research has witnessed a paradigm shift away from an exclusive focus on the human genome and its functions and towards a greater understanding of our fellow travelers: the microorganisms that live within and on our bodies. Formerly studied exclusively in terms of their pathogenic and disease-promoting potential, bacteria, viruses, archaea, fungi and other microorganisms are now being examined in a completely different light – as commensals critical for the homeostasis of the host. Such studies have not only opened a new platform for research into disease pathophysiology, but also revealed the potential for developing new management strategies for several disease states and syndromes. Much of the progress in this field can be attributed to major and ever evolving developments in technology, which now permit the rapid and complete identification of all of the bacterial inhabitants of a given locus.[1] With these technologies comes new terminology, a terminology that will be new to many and confusing to some. To facilitate the reader's access to the literature on this field a list of the more commonly used terms and their definition is provided in Table 1. You will notice one striking omission from this list: "flora". This term, which dates from the time when bacteria were included in the plant kingdom, has now been largely abandoned and replaced by "microbiota".

The results of the human genome project were a surprise to researchers with the discovery of only 20-25000 genes, about one fifth of what was expected.[2] So, to look for the missing pieces in the puzzle, other sources of genetic information were explored; giving birth to the concept of the microbiome and ultimately to the human microbiome project.[3] It turns out that microbes are not "mere bugs in our system" but in fact are playing a very important symbiotic role. Insights into the function of these organisms have been provided, in the first instance, by an interrogation of their genome, through metagenomics and thereby, to

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Table 1. Important Terms and Their Definition

Microbiota	The assemblage of microorganisms (bacteria, archaea or lower eukaryotes...) present in a defined environment.
Microbiome (Human)	The full complement of microbes (bacteria, viruses, fungi, and protozoa), their genes, and genomes in or on the human body.
Metagenomics	The study of the gene content and encoded functional attributes of the gut microbiome in healthy humans.
Metabonomics:	Quantitative measurement of the multiparametric (time-related) metabolic responses of complex systems to a pathophysiological stimulus or genetic modification; often used synonymously with metabolomics.
Commensal	Organism participating in a symbiotic relationship, in which one species derives some benefit while the other is unaffected.
Prebiotic	A substance that (1) is resistant to gastric acidity, to enzymatic hydrolysis, and to gastrointestinal absorption (i.e., not hydrolytically digestible); (2) is fermented by cecal-colonic microbiota; and (3) selectively stimulates growth and/ or activity of those bacteria that contribute to colonic and host health.
Probiotics	Living microorganisms that when administered in adequate amounts confer a health benefit on their host.

the identification of genes linked to certain biological functions. Correlations with function have been taken a step further through the application of metabolomics and other techniques that identify the products of bacterial synthetic and metabolic processes.[4,5]

While the microbiome of each individual is quite distinct at the level of individual bacterial strains, data from a European consortium indicated that at a higher level of organization, some general patterns can be identified across populations.[6] They identified three broad groupings driven by the predominance of certain species: *Prevotella*, *Bacteroides* and *Ruminococcus*. Enterotype prevalence seemed independent of age, body mass index or geographic location but may be driven by differing dietary habits. Indeed, the importance of diet in shaping, both in the short- and in the long-term, the composition of the microbiome is now a subject of active study and the impact of dietary changes on the microbiome may well have been underappreciated in former studies.[7]

The Microbiome in Health

At birth the 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.[1,8] The mode of delivery (vaginal birth vs. caesarean section), diet (breast milk vs. formula), level of sanitation and exposure to antibiotics all influence the development of the infant's microbiome.[8-11] By 2 to 3 years of age, the child's microbiota fully resembles that of an adult in terms of composition.[1,12,13]

Thereafter the microbiota is thought to remain relatively stable until old age when changes are seen

possibly related to alterations in digestive physiology and diet.[13-15] It needs to be emphasized that there are relatively few longitudinal studies.

1. What Regulates 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, though certainly not sterile, contain relatively small numbers of bacteria in healthy subjects.[1,16] 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 microbiota changes dramatically. Concentrations of 10^{12} CFU/mL, or higher, may be found; comprised mainly of anaerobes such as *Bacteroides, Porphyromonas, Bifidobacterium, Lactobacillus and Clostridium*, with anaerobic bacteria outnumbering aerobic bacteria by a factor of 100-1000:1. The predominance of anaerobes in the colon reflects the fact that oxygen concentrations in the colon are very low; the microbiota has simply adapted to survive in this hostile environment.

Though most studies of the human gut microbiota have been based on analyses of fecal samples it must be pointed out that at any point along the gut differences are also evident between bacterial populations resident in the lumen and those adherent to the mucosal surface. These mucosa-associated bacterial species and strains will not be accurately represented in fecal samples, a major limitation of this approach. 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 the products of digestion.

Antibiotics, whether prescribed or in the food chain, have the potential to profoundly impact the microbiota.[16] In the past, it was believed that these effects were relatively transient with complete recovery of the microbiota occurring very soon after the course of antibiotic therapy was complete. However, while recent studies have confirmed that recovery is pretty rapid for many species, some species and strains show more sustained effects.[17] Furthermore, antibiotic exposure and related disruptions of the microbiome may be especially critical in infancy as the microbiome develops.

2. The Functions of the Microbiome

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 the mucosa-associated immune system, immunological tolerance, epithelial and barrier function, motility and vascularity. The resident commensal microbiota continues to contribute to such homeostatic functions during life as pathogen exclusion, immunomodulation, upregulation of cytoprotective genes, prevention and regulation of apoptosis and maintenance of barrier function.[18]

The sophistication of the relationship between the microbiota and its host is elegantly illustrated by the manner in which the immune system of the gut differentiates between friend and foe when it encounters bacteria.[19] At the epithelial level, for example, a number of factors may allow the epithelium to "tolerate" commensal (and thus probiotic) organisms. These include the masking or modification of microbial associated molecular patterns that are usually recognized by pattern recognition receptors (PPR's), such as Toll-like receptors (TLR's)[20] and the inhibition of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) inflammatory pathway.[21] Responses to commensals and pathogens may also be distinctly different within the mucosal and systemic immune systems. For example, commensals such as *Bifidobacterium infantis* and *Faecalobacterium prasunitzii*, have been shown to differentially induce regulatory T cells (Tregs) and result in the production of the anti-inflammatory cytokine, IL-10.[22] Other commensals may promote the development of T helper cells, including T_H17 cells and result in a controlled inflammatory response which is protective against pathogens in part, at least, through the production of IL-17.[23] The induction of a low-grade inflammatory response ("physiological" inflammation) by commensals could be seen to "prime" the host's immune system to deal more aggressively with the arrival of a pathogen.[24] It is also now evident that host-microbe immune interactions are bidirectional; innate immune responses can shape

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the microbial ecology of the gut and this, in turn, can influence the development of disease susceptibility in the host.

Some of the metabolic functions of the microbiome have been known for years: the ability of bacterial disaccharidases to salvage unabsorbed dietary sugars, such as lactose, and alcohols and convert them into short-chain fatty acids (SCFAs), the synthesis of nutrients and vitamins, such as folate and vitamin K, the deconjugation of bile salts[25] and metabolism of certain drugs (e.g. sulfasalazine). Now a fuller picture of the metabolic potential of the microbiome is being revealed and includes the production of other chemicals, including neurotransmitters and neuromodulators, which can modify other gut functions, such as motility or sensation, or even influence the development[26] and function[27] of the central nervous system, thereby leading to the concept of the microbiota-gut-brain axis.[28-30]

The Gut Microbiota and Disease

The idea that the bacterial contents of the gastrointestinal tract could contribute to symptoms and disease is not a new one; the role of enteric bacteria in hepatic encephalopathy was described over 50 years ago and several other human ailments have been clearly defined as originating from a disturbed microbiome and/or how it interacts with the host. Well accepted examples are listed on Table 2. The availability of high-throughput sequencing techniques, as well as exciting data from animal experiments, has spurred a host of studies of the microbiome in almost every known gastrointestinal, liver and pancreaticobiliary disease. Based on such studies, a role for the microbiome and/or host-microbiome interactions has been proposed for a long list of diseases and syndromes, some of which are listed on Table 3. While, in some instances, such as inflammatory bowel disease (IBD), 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 describe a mere association and no conclusions can be drawn with respect to causation.

With respect to disease causation, the 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 are, potentially, doomed to failure.

Table 2. Microbiota and Disease: gastrointestinal, liver and pancreatico-biliary disorders where the assemblage of microorganisms (bacteria, archaea or lower eukaryotes) present in a defined environment where relationships to the microbiome and/or microbiome-host interactions are well established.

Enteric Infections and Infestations

Helicobacter pylori

Antibiotic-associated diarrhea

- ***Clostridium difficile*-associated Disease (CDAD)**

Small Intestinal Bacterial Overgrowth

Complications of Liver Disease

- **Portal-Systemic Encephalopathy**
- **Spontaneous Bacterial Peritonitis**

Biliary and Pancreatic Sepsis

Helicobacter Pylori

Helicobacter Pylori, one of the most studied of all bacteria, provides a beautiful illustration of host-microbe interactions with the disease phenotype resulting from infection with this fascinating organism reflecting complex interactions between bacterial properties, host factors and other environmental influences, including the resident gastric microbiome. For example, certain Bifidobacterium strains display anti-Helicobacter effects through the production of antimicrobial peptides.[31]

Diarrheal Illness

Infectious diarrheas, still a major cause of morbidity and mortality worldwide, represent an overwhelming assault on the commensal microbiome and the host. Pathogens have evolved a number of strategies to survive in the gut and evade immunological and physiological responses by the host. Here again, microbe-host responses play a critical role; some bacteria take advantage of the host's inflammatory response to its presence to create

a favorable environment that allows them to outgrow resident microbes. For example, gastroenteritis due to *Salmonella typhi* has been well studied in terms of the genetic adaptations of the pathogen and the role of the host immune system in determining disease outcome.

Antibiotic-associated diarrhea and its most concerning manifestation, *Clostridium difficile*-associated disease (CDAD), 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.[32] Evidence suggests that the predilection to *C. difficile* illness is largely a function of how resilient the indigenous microbiota is following an antibiotic assault, with some bacterial communities being better able to recover than others. The management of CDAD is now complicated by the emergence of hypervirulent strains and an ever-increasing rate of recurrence following initial treatment with metronidazole or vancomycin. Recurrence rates of 25 percent or more are now commonly reported. The role of an indigenous healthy microbiome is perhaps most dramatically illustrated by the overwhelming success of fecal microbiota transplantation (FMT) in the management of recurrent CDAD.

Irritable Bowel Syndrome (IBS)

Several strands of evidence suggest a role for the gut microbiota in IBS.[33] First and foremost among these is the clinical observation that individuals can develop IBS *de novo* following exposure to enteric infections and infestations, post-infectious IBS (PI-IBS).[34] More contentious has been the suggestion that IBS subjects may harbor small intestinal bacterial overgrowth (SIBO).[35] More indirect evidence for a role for the microbiota can be gleaned from some of the metabolic functions of components of the microbiota. Thus, changes in bile salt deconjugation could, given the effects of bile salts on colonic secretion, 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. Sequencing studies have shown that IBS patients, regardless of subtype, do exhibit a fecal microbiota that is clearly different from control subjects.[36] Such studies have demonstrated reduced microbial diversity in IBS[37] and the existence of different IBS subgroups[38] defined by the relative proportion of the two major phyla, *Firmicutes* and *Bacteroidetes,* as well as significant changes at species and strain level.[38,39] The primacy of these microbial shifts and their potential to disturb mucosal or myoneural function in the gut wall, impact on the brain-gut axis, or induce local or systemic immune responses remains to be defined.

Table 3. Microbiota and Disease: Gastrointestinal, Liver and Pancreatico-Biliary Disorders Where a Role for the Microbiota and/or Host-Microbiome Interactions Has Been Postulated

- Functional Dyspepsia (FD)
- Diverticulitis
- Necrotizing Enterocolitis (NEC)
- Inflammatory Bowel Disease (IBD)
- Ulcerative Colitis (UC)
- Crohn's Disease
- Pouchitis
- Irritable Bowel Syndrome (IBS)
- Celiac Disease
- Non-Alcoholic Fatty Liver Disease (NAFLD)
- Alcoholic Liver Disease
- Intestinal Failure-Associated Liver Disease
- Primary Sclerosing Cholangitis (PSC)
- Primary Biliary Cirrhosis (PBC)
- Pancreatitis

Obesity, the Metabolic Syndrome and Related Disorders

A considerable body of basic research suggests an important role for the microbiota in the development of obesity and related disorders, such as the metabolic syndrome.[40,41] Qualitative changes in the gut microbiota have also been identified in man but findings have been less clear-cut. Nevertheless, a microbial signature predictive of the development of type II diabetes has also been identified and FMT was shown to restore insulin sensitivity in a small study among individuals with the metabolic syndrome.[42] Fundamental to all theories of the role of the microbiota in these disorders is the concept that a shift in the composition of the microbiota towards a population where bacteria that are more avid extractors of absorbable nutrients, results in the availability of these nutrients for assimilation by the host; thereby, contributing to obesity.[40]

Colorectal Cancer

Recent studies have identified specific signatures in the gut microbiome associated with colorectal cancer (CRC) and suggested that the microbiome may serve as a valuable screening tool; the efficacy of this approach in clinical practice has yet to be demonstrated. While microbiome-based analyses, on their own, can detect precancerous and cancerous lesions, combining such data with body mass index, a known clinical risk factor of CRC, and occult blood testing, provided an excellent discrimination between healthy individuals to those with malignant and premalignant lesions.[43]

While a disturbed microbiota has been linked with CRC, defining a causal link has proven more problematic. In recent years, research has focused on identifying bacterial species or strains that are particularly linked with CRC.[44] Two bacteria in particular, Fusobacterium nucleatum and Escherichia coli, have been consistently associated with CRC. Proposed pathways to cancer formation related to bacteria have included bacteria- induced chronic inflammation leading to cell proliferation or the direct effects of bacterial virulence factors inducing tumor formation.[45]

Inflammatory Bowel Disease (IBD)

A considerable body of experimental and clinical evidence indicates that the microbiota and microbiota-host interactions are critical to the pathogenesis of IBD.[46] Defining the precise nature of the fundamental pathophysiology has proven more challenging; is it an abnormal microbiota, an abnormal host immune response to a normal microbiota or some combination of these factors? There is some evidence for the presence of a disturbed microbiota in IBD but results are not consistent. For example some studies demonstrated that patients with Crohn's disease (CD; either colonic or ileal) exhibited microbiota profiles distinctly different from those of healthy controls or patients with ulcerative colitis (UC). Furthermore, the fecal microbiota in patients with ileal CD differed from that in patients with predominantly colonic disease.[47] In contrast, data from a twin study suggested that the microbiome was abnormal in UC also.[48] Several factors contribute to sorting out the role of the microbiome in IBD: the heterogeneity of the disease population, diet, medications and disease activity. For example, it is distinctly plausible that changes in the microbiome seen in IBD could reflect the consequences of inflammation and have nothing to do with causation. Longitudinal studies of the gut microbiota throughout the course of the disease are needed.

Liver Disease and its Complications

That the microbiota-gut-liver axis plays an important role in the occurrence of infectious and noninfectious complications of liver disease is well established. More recent is the proposal that the microbiota could be involved in the pathogenesis of liver diseases, such as non-alcoholic liver disease (NAFLD).[49] From a considerable body of experimental and some clinical data some common themes have emerged. Thus, a disturbed microbiota (small intestinal overgrowth and/or qualitative changes in the microbiota), impaired gut barrier function and the host immune response have been shown to conspire to impact on liver metabolism (contributing to lipogenesis, for example), promote inflammation and even contribute to the progression to fibrosis, cirrhosis and hepatocellular carcinoma. The microbiota has also been implicated in alcoholic liver disease. Alcohol impairs the host immune response.[50] and its metabolites can conspire with lipopolysaccharide (LPS) produced by Gram-negative bacteria to induce liver injury.[51] The microbiota also contributes to alcohol-related liver injury by promoting the growth of endotoxin-producing gram-negative bacteria in the gut and increasing intestinal permeability.

The role of antibiotic therapy is well established in the

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prevention and management of hepatic encephalopathy and infectious complications of liver disease.[52] Now microbiota-modulating strategies are being explored in the management of liver disease *per se*. For example, the probiotic organism *Lactobacillus rhamnosus,* has been shown to promote gut homeostasis by modulating the growth of Gram- negative bacteria[53] and restoring intestinal barrier integrity; as a consequence liver fat content and circulating levels of pro-inflammatory cytokines are reduced.[54,55]

Therapeutic Modulation of the Microbiome

While it is undoubted that food is the primary modulator of the microbiome, it is not the only one. Specifically, extensive antibiotic use in modern animal husbandry exerts a selective pressure for antibiotic resistance that eventually spreads to the human microbiome. Because of the rapid and efficient transfer of resistance genes from one bacterium to another, even nonpathogenic (so-called commensal) bacteria can carry and express resistance genes.

Probiotics and prebiotics aim to confer a health benefit by modulating the microbiome. Prebiotics selectively stimulate the growth and/or activity of bacteria that contribute to colonic and host health.[56] Probiotics may provide benefits through the multiple aforementioned mechanisms whereby the normal commensal microbiota interacts with the host. While the traditional concept of probiotics is based on the functions of live organisms, it is evident that dead bacteria, bacterial components or bacterial metabolites are biologically active. For example, probiotics have the potential to either stimulate or suppress host immunity via microbe-derived immunomodulatory molecules.[57] A complete discussion of the use of probiotics in man is beyond the scope of this review. Suffice it to say that, given the current regulatory climate, major quality control issues surround the probiotic market. At the very least a probiotic should be characterized at genome level and should have been demonstrated to survive passage through the digestive tract to its desired site of action. Furthermore, clinical claims should be supported by high quality clinical trial data. Although there is no such thing as zero risk, probiotics are generally regarded as safe and truly probiotic-related adverse events in healthy individuals and those seen in an ambulatory care setting have been vanishingly rare.[58]

CONCLUSION

These are exciting times in microbiome research. A field that simply did not exist a few years ago has exponentially expanded to become one of the hottest in all of biomedicine. As techniques develop, become more rapid and less costly, the delineation of the true extent of the role of our bacterial fellow travelers in health will soon be realized. In terms of disease states, while many tantalizing associations have been described, defining causation will take some time given the heterogeneity of many disease populations, the dynamics of the microbiota over time, the bidirectional nature of interactions between the host and the microbiome and the impact of so many confounding factors. There is much to be done. ■

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