LECTURE

Gut Microbes: From Bugs to Drugs

Fergus Shanahan, MD, FACG[1]

A poorly appreciated truism is that the information contained within the mammalian genome is insufficient for full development of several organ systems, notably the gut, immune system, and other sensory organs. The required information is derived from the environment, including the microbial environment. This suggests that the microbiota is a source of regulatory signals, some of which may be suitable for exploitation for therapeutic purposes. Indeed, it could have been deduced from comparative studies of germ-free and conventionally colonized animals almost half a century ago that the gut microbiota influences the development and maturation of the digestive and immune systems. In some instances, the signals involved have recently been defined molecularly. This opens the possibility of a "bugs to drugs" program of discovery, in which the gut ecosystem is explored as a repository from which bioactives or novel drugs might be mined and translated to human health care. Specific examples of mining microbe–microbe interactions, host–microbe interactions, and host–microbe–dietary interactions have immediate clinical implications. The future of drug discovery in gastroenterology is likely to reside in the lumen!

Am J Gastroenterol 2010; 105:275–279; doi:10.1038/ajg.2009.729; published online 12 January 2010

INTRODUCTION

Drivers of a resurgence of interest in the mighty microbe

This article is based on the David Sun lecture of the annual meeting of the American College of Gastroenterology, San Diego, CA, USA on 24 October 2009.

A convergence of clinical observations and technological advances has accelerated interest in the alimentary microbiota, both as a therapeutic target and as a repository from which new drugs may be mined. Among the key drivers of the resurgent interest in commensal microbiota was the discovery of *Helicobacter pylori* as a cause of peptic ulcer disease. A sobering lesson of this story was that decades of epidemiological observations missed the critical involvement of a transmissible agent in such a common disease. This highlights the limitations of traditional epidemiology, which may be too restrictive and distracted by epiphenomena or "risk-factor epidemiology", without due rapprochement with potential disease mechanisms (1). Of course, generations of biologists also missed the microbial contribution to the disease. Was this due to a paucity of convergent thinking or "hybrid science" and the need for investigators capable of thinking across the boundaries of traditional disciplines? Another lesson from this episode was that seemingly complex and heterogeneous disorders may be infectious in origin, and although multifactorial, can be solved by taking out a key component of the pathogenesis.

However, the most important lesson of the *Helicobacter pylori* story was that the solution to some chronic human diseases can never be found by research focused exclusively on the host, without due regard for interactions with environmental microbiota. It is unlikely that peptic ulcer disease represents the only example of this, and it may be time to apply the lesson to other modern scourges, such as irritable bowel syndrome or colon cancer.

Another stimulus for renewed interest in the gut microbiota was the discovery that genetic risk factors for Crohn's disease are mutant genes that code for sensors of the microbial environment (CARD15/NOD2) or for regulators of host immune responses to that environment (autophagy, IL-23R) (2,3). The clinical significance of these observations has been underscored by increasing evidence linking a modern lifestyle with changes in the alimentary microbiota in early life and thence to risk of immunoallergic disorders (4). Thus, the commensal microbiota is a regulator of immune development, and many of the elements of modern lifestyle may represent proxy markers of microbial exposure during mucosal immune maturation.

The collective microbial genome in the gut (microbiome) contains 10- to 100-fold more genes than the human genome and provides the regulatory signals for immune and gastrointestinal maturation (5–7). This raises the possibility that in susceptible individuals, the presence or absence of some components of the microbiota may be an epigenetic risk factor for inappropriate or excessive immune responses and the development of inflammatory bowel disease.

A pivotal technological advance has been the circumvention of a major impediment to microbial research—inability to culture the majority of microbes in the gut. This has been achieved by new molecular techniques, notably metagenomics and compositional sequencing (8), which have enabled the study of mixed communities of microbes in the gut, and revealed greater diversity than previously imagined. More importantly, the gut microbiota has been

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Received 12 November 2009; accepted 9 December 2009

implicated not only in diseases of the gastrointestinal tract but also in several extraintestinal disorders, such as obesity, diabetes, and metabolic syndrome (9–12).

Manipulating the microbiota—myths and misperceptions

The intestinal microbiota is both a health asset (for maintenance of intestinal homeostasis) and an occasional liability (as a contributor to the pathogenesis of disease in susceptible hosts). This provides the rationale for therapeutic manipulation of the microbiota, which may be defined simply as any intervention to enhance microbial assets or to offset liabilities. Although manipulating the microbiota by natural strategies, such as consumption of probiotics or otherwise, is conceptually appealing to many patients, clinicians will increasingly need an authoritative response to some of the myths and misunderstandings surrounding the notion of therapeutic manipulation of the microbiota.

Sources of potential misperception include the following:

(i) *Terminology:* When is a so-called "probiotic" really a probiotic? As there is no legal definition of the term, consumers and prescribers need to be cognizant of guidelines on the scientific basis of health claims for commercially available products. Furthermore, restrictive definitions may have outlived their usefulness and should not exclude the potential benefit of strategies that involve dead organisms, components of organisms, or metabolites of microbes. For this reason, the umbrella term, pharmabiotic, to encompass the wider potential use of functional microbes and their products may be preferable to terms such as probiotics, prebiotics, synbiotics, and postbiotics. An alimentary pharmabiotic is any material with a potential health benefit that can be mined from host–microbe–dietary interactions (13).

(ii) *Expectations:* The efficacy of probiotics, as with most naturally occurring remedies, is likely to be modest and nonuniform. They should be considered as supplements, not substitutes, for conventional therapy. As alluded to already, the impact of intestinal bacteria on the developing immune system is greatest at the earliest stages of postnatal life. Therefore, the notion of manipulating the microbiota in adulthood as a therapeutic strategy for disorders such as inflammatory bowel disease may be futile—too late to achieve substantial efficacy. This may explain the apparent disparity in the efficacy of probiotics in attenuating animal models of inflammatory bowel disease and the disappointing results to date in humans with the same organisms (14).

(iii) *The folly of considering probiotics exclusively in generic terms:* This is as simplistic and absurd as the notion of administering "pills" or "tablets" without regard for the nature of the active ingredient and the intended effect. Clinical results with one probiotic cannot be extrapolated to another or to a separate clinical indication. For example, the specificity of probiotic action has been molecularly defined in the case of protection against *Listeria* infection with *Lactobacillus salivarius UCC 118*, in which the mechanism of action has been fully explained by the production of an antimicrobial peptide by the probiotic (15). However, other probiotics do not necessarily exhibit the same mechanism of action, and this is not the mechanism by which the same probiotic protects against other pathogens. Thus, protective mechanisms may be strain specific and disease specific. Indeed, the clinical importance of good science with careful strain selection for specific indications has been highlighted by a report of an adverse outcome in a trial of a probiotic preparation in patients with acute pancreatitis (16).

(iv) *How can so few influence so many?* Skeptics often proclaim that probiotics are unlikely to have a meaningful impact on the intestinal ecosystem because the usual daily consumption (10^9–10^{12} organisms) is a tiny fraction of the total commensal bacterial population resident within the gastrointestinal tract. However, elegant studies with gnotobiotic mice have shown that the resident bacteria adapt to the introduction of a probiotic species and to host dietary changes with alterations in gene expression and metabolic behavior (17,18).

(v) *A neglected interaction—dietary manipulation of the microbiota*: Unfortunately, the impact of diet and nutrition on disease or wellness is often taken more seriously by patients than by their clinicians. However, the greatest environmental influence on the commensal microbiota is dietary. Dietary poly/oligosaccharides (prebiotics) have a well-documented influence on the microbiota, but less well recognized is the potential role of dietary fat on microbial metabolism. Compelling evidence has shown that the gut microbiota is an environmental regulator of fat storage in humans (9), and more recently, microbial metabolism of specific dietary fatty acids in the gut has been shown to influence the composition of bioactive fatty acids in the adipose tissue of the host (19). This, in turn, influences the immunoinflammatory response, a finding that is consistent with emerging evidence of the proinflammatory nature of excess adipose tissue in humans. Indeed, the possibility that dietary changes associated with socioeconomic development might contribute to the changing epidemiology of inflammatory bowel disease and immunoallergic diseases has been addressed recently (4). It may be particularly relevant that the increased incidence of inflammatory bowel disease over recent decades in Japan correlates closely with changes in dietary fat, particularly animal fat and n-6 polyunsaturated fatty acids (20).

From manipulation to mining

Regardless of the unfulfilled potential of therapeutic manipulation of the microbiota in certain clinical disorders, the prospect of mining host–microbe–dietary interactions for novel drug discovery is emerging as a more intriguing reality. This arises at a time

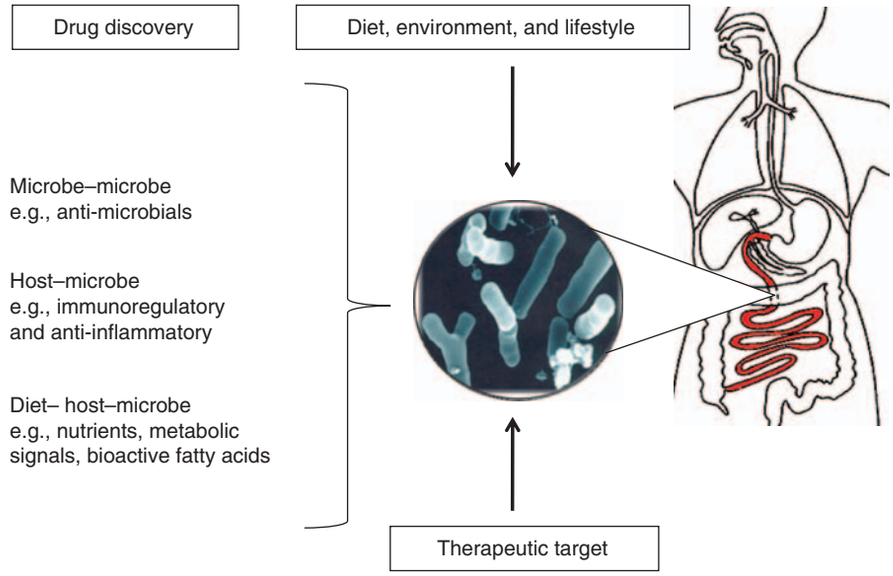

Figure 1. Schematic representation of the therapeutic potential of the gut microbiota. The microbiota is influenced by diet and lifestyle factors, and has become a target for drug therapy in various disorders. However, it also offers new opportunities as a repository of bioactives from which novel drugs may be mined.

when traditional approaches to drug development by pharmaceutical companies are becoming more difficult, and seem to be in decline, particularly in gastroenterology (21,22). Contributory factors include high development costs, regulatory constraints, and safety concerns. In addition, "big pharma" now struggles with the realization that the era of the blockbuster, one-size-fits-all drug may be eclipsed by a more personalized approach to therapeutics. Furthermore, large fortunes have been expended by the pharmaceutical industry developing synthetic drugs; yet, many of the most versatile and useful drugs have been derived from living material in the wider environment (23). Perhaps the inner world of the gut microbiota may offer a new frontier, a more natural and accessible opportunity for novel drug discovery (24).

The premise or biological plausibility of such a proposition is based on studies of life without bacteria. From comparisons between germ-free animals and conventionally colonized controls, it is clear that the gut microbiota must be a source of regulatory signals influencing the maturation of the gut, immune system, and other organs (5,7). Defining the molecular identities of these microbial-derived signals creates the potential to mine the microbiota for novel drug discovery or for new bioactives suitable as functional food ingredients. Bioprospecting the gut microbiota for such therapeutics is already being translated into the clinical arena. Representative examples follow (**Figure 1**).

(i) *Host–microbe interactions*: The specific composition of the gut microbiota has been shown to have a profound impact on immunological differentiation. This has been most strikingly shown in the case of the balance of effector and regulatory cell (T_H17/Treg) activity (25). In the absence of bacteria, the mucosal and systemic limbs of the immune system are structurally and functionally defective (26,27). As this can be restored on colonization with commensal microbiota, it follows that the luminal microbiota must be a source of immunomodulatory signals. It is well established that bacteria are a source of a diverse variety of immunomodulatory molecules, including bacterial nucleic acids and oligonucleotides containing hypomethylated CpG dinucleotides that are ligands for toll-like receptor-9 (28). In addition, several microbial proteins and peptides with cytoprotective or anti-inflammatory activities have also been identified (29,30). Remarkably, colonization with a single bacterial commensal strain has been sufficient to show the impact of microbial signaling on the expression of host genes controlling gastrointestinal structure and function. In one instance, the microbial signal has been molecularly defined as a single immunomodulatory polysaccharide derived from *Bacteroides fragilis,* which can correct mucosal and systemic immune defects in germ-free mice (27). The therapeutic potential of this observation is highlighted by the use of the same polysaccharide to prevent intestinal inflammatory disease in a murine model (31).

(ii) *Dietary–microbe–host interactions*: The most important environmental or lifestyle influences on commensal microbiota are probably diet and nutrition. The complexity of these interactions is underscored by evidence of the controlling influence of the microbiota on fat storage or quantity (9). More intriguingly, fat quality has been shown to be regulated by the gut microbiota. Thus, microbial metabolism in the gut (in the presence of appropriate substrate of dietary origin) has a profound influence on

the composition of bioactive fatty acids, such as conjugated linoleic acid and eicosapentanoic acid, in adipose and other host tissues (19). Thus, an ingested commensal or food-grade organism may interact with dietary lipid substrate and have an impact on the bioactive fatty acid composition of extraintestinal organs in the host. As the fatty acid composition of cell membranes may set the tone for pro- or anti-inflammatory cytokine release, these observations offer new opportunities for dietary manipulation of immunoallergic disorders (19).

(iii) *Microbe–microbe interactions*: As microbe–microbe signaling contributes to the regulation of bacterial numbers within different niches of the alimentary tract, the prospect of mining interbacterial signaling molecules represents a strategy for discovery of novel antibiotics. An example is the therapeutic exploitation of bacteriocins. Bacteriocins are a family of antimicrobial peptides to which the producer organism has specific resistance and which inhibit the growth of other, often closely related bacteria (32). A bacteriocin-producing strain of *Streptococcus salivarius* has been reported to be of use in the management of halitosis (33). More importantly, the broad-spectrum bacteriocin, lacticin 3147, has been shown to have activity *in vitro* against *Clostridium difficile*, with potency comparable with that of currently used conventional antibiotics, metronidazole and vancomycin (34). A confounding impediment to wider clinical application is the problem of enzymatic degradation in the lumen of the bowel, but this can be overcome by pharmaceutical strategies, such as encapsulation and sustained- or delayed-release formulations. In addition, a systematic search for a narrow-spectrum bacteriocin with relative specificity for *C. difficile* has led to the discovery of a new class of bacteriocin, thuricin, within our center (Rea M *et al.*, unpublished). This is particularly promising and timely, in view of the emergence of highly virulent forms of *C. difficile*-associated disease and the increasing resistance of the organism to available antibiotics (35).

CONCLUSION

What mad pursuit bioprospecting the commensal microbiota might once have seemed! However, today the scope for harnessing the power of the gut microbiota is no longer limited by technology, but primarily by our imagination. Given the influence of the microbiota on the developing immune system, one might predict that some day it will become routine to control the composition of the microbiota colonizing the human neonate. This may have particular relevance to low birth weight babies born with an immature digestive tract and incompletely developed immune system. Indeed, the concept of immunization, usually considered in the context of protection against pathogens, may soon be applied to oral immunization with antigens derived from intestinal commensals that coevolved with humans over the millennia ("old friends"). Manipulating and mining the microbiota promises much, but this will be realized with greater understanding of the diversity and complexity of the normal microbiota in different populations with different lifestyles. This field of endeavor is in its infancy, but the earliest steps have been taken. Where lies the future? It is in the lumen! Onward!

CONFLICT OF INTEREST

Guarantor of the article: Fergus Shanahan, MD, FACG.
Specific author contributions: Manuscript preparation: Fergus Shanahan.
Financial support: Fergus Shanahan is supported in part by Science Foundation Ireland and by grants from the Irish Department of Agriculture and Food, the Higher Education Authority of Ireland, and the European Union.
Competing interests: He is a stockholder of Alimentary Health Ltd., has consulted for Procter and Gamble Co., and has received research grants from GlaxoSmithKline Ltd. The content of this document was neither influenced nor constrained by these facts.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ The gut microbiota contributes to host defense but in susceptible individuals may participate in the pathogenesis of inflammatory and other diseases.
- ✓ The gut microbiota is a legitimate therapeutic target in some diseases.
- ✓ The gut microbiota influences the development and maturation of the digestive and immune systems.

WHAT IS NEW HERE

- ✓ The gut microbiota is a source of regulatory signals for immune development—such signals may be mined for novel immunoregulatory drug discovery.
- ✓ Microbe–microbe interactions in the gut represent a repository for novel antibiotic discovery.
- ✓ Manipulation of host–diet–microbe interactions in the gut is an untapped therapeutic opportunity.

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Received 12 November 2009; accepted 9 December 2009