Ecology and Medicine Converge at the Microbiome-Host Interface

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ABSTRACT The human body is home to a dense and diverse population of bacteria, viruses, and eukaryotes, collectively termed the microbiome. Research on host-microbiome interactions continuously demonstrates the importance of this microbial community to human physiology and its involvement in a myriad of diseases. This, in turn, sparks great interest in developing means for beneficially modulating the microbiome, such as fecal microbiome transplantation and probiotics. However, these interventions show mixed efficacy in clinical trials and raise safety concerns. How these exogenous microorganisms interact with the microbiome might underlie the efficacy and safety of these therapeutics, yet the signaling mechanisms mediating microbe-microbe interactions between human-dwelling commensals are poorly understood. In this commentary, we discuss known and putative mechanisms of interactions between commensals in the gut and how they can be harnessed for improving microbiome-targeting therapeutics and facilitating translation of microbiome research to the clinic.

KEYWORDS FMT, microbiome, antibiotic resistance, microbial ecology, postantibiotic effect, probiotics

MICROBIOME INTERACTIONS WITH MICROBIAL THERAPEUTICS SHAPE EFFICACY AND SAFETY

The human gut is home to a dense and diverse population of bacteria, archaea, fungi, viruses, and protists. These microbes cooperate, cross-feed, compete, antagonize, and prey on each other and, in doing so, form a complex microbial community that is critical for the development and well-being of their human host. These microbe-microbe interactions are part of the ecological forces that, along with host and environment factors, drive the formation of a health- or disease-associated microbiome.

In addition to interactions among microbes already entrenched in the gut, the resident microbiome interacts with exogenous microorganisms to modulate their engraftment potential and consequently their effect on the host. The mechanisms through which the microbiome confers colonization resistance against pathogens are a major focus of microbiome research (1). However, exogenous microbes can also be beneficial to the host’s health. Fecal microbiome transplantations (FMT) from healthy donors, defined bacterial consortia (probiotics), and phage therapy aim to produce a health benefit by administering exogenous microorganisms that target the host or its microbiome. An emerging theme pertaining to the efficacy of these therapeutics is the importance of their successful engraftment in the recipient’s gut.

FMT is currently used for treating Clostridioides difficile infections and is extensively studied as a therapeutic for a myriad of other conditions. The high and reproducible efficacy of FMT in recurrent C. difficile infections can be attributed to the low-diversity microbiome, which is more permissive to colonization by exogenous microorganisms. In contrast, the efficacy of FMT in ameliorating other conditions, where the exogenously...
introduced microorganisms encounter an entrenched dysbiotic microbial community, remains a challenge (2), with some studies showing only transient health benefits or none whatsoever. The clinical benefits of FMT are also heterogeneous, either between patients in the same study or between trials evaluating FMT efficacy for the same condition. Several factors have been suggested to contribute to the limited efficacy and heterogeneity in results, including engraftment success, which may depend on the pretransplantation microbiome (2). Similar to FMT, health benefits of probiotics are heterogeneous among studies and individuals (3). Successful engraftment of the exogenous probiotic strains also varies between individuals and is associated with permissiveness of the presupplementation microbiome (4–6).

Whether engraftment is necessary for probiotics to modulate human health is debated, as even inactivated bacteria or their purified cell components or metabolites can produce an effect in vivo. Nonetheless, in our work, lack of probiotic colonization in the gastrointestinal mucosa resulted in a limited probiotic effect of on the microbiome and immune pathway expression, two mechanisms through which probiotics exert a beneficial effect (6). Furthermore, we recently reported that probiotics can reduce the number of antibiotic resistance genes (ARG) in the gut, but only in colonization-permissive individuals (7). Similarly, a beneficial effect of probiotics on irritable bowel syndrome (IBS) and murine models of colitis and depression was associated with probiotic colonization and distinct presupplementation microbiome (3). It is possible that some probiotic mechanisms of action can transiently occur even in the absence of engraftment, such as secretion of bacteriocins, or those that are induced following antigen-presenting cells sampling probiotic bacteria from the lumen. In contrast, a clinical benefit might require mucosal colonization if the underlying mechanisms involve binding of host receptors on mucosa-associated cells, or microbiome modulation (3). Thus, understanding the interactions between the endogenous microbiome and the exogenous community underlying engraftment permissiveness could contribute to improved clinical efficacy of probiotics and FMT.

Notably, enhanced probiotic colonization may not necessarily be beneficial. We previously reported that a course of antibiotics considerably improves probiotic colonization in the human gastrointestinal mucosa. In turn, probiotics delay recovery of the microbiome diversity to the preantibiotic state, a process associated with the secretion of currently unidentified soluble molecules (8). As a result, the antibiotic-associated expansion of ARG in the gut persists and is even further exacerbated in probiotic-supplemented individuals (7). While additional clinical implications of delayed microbiome recovery remain to be determined, identifying the mechanisms through which exogenous bacteria antagonize commensals could improve the safety profile of microbial therapeutics.

MECHANISMS OF MICROBIAL INTERACTIONS AND THEIR TRANSLATIONAL ASPECTS

Microbes utilize a wide array of mechanisms for intra- and interspecies interactions (Fig. 1). In the context of gut commensals, much of our knowledge is related to metabolic interactions: either cross-feeding, i.e., the secretion and consumption of metabolites by other microbial cells (9), or competition over nutrients (1). In addition, horizontal gene transfer (HGT) through transformation, conjugation, or transduction is common in the human microbiome and may reflect adaptation to the host’s lifestyle (10). In comparison, many other types of microbial interactions that were demonstrated in vitro, or in environmental ecosystems, have not been explored in the gut or have been studied exclusively in pathogen-commensal interactions in this niche. For example, in addition to conjugation and transduction, multiple gut commensals can produce extracellular vesicles carrying diverse cargo. These have mostly been studied for their effect on the host but were shown to mediate bacterial interactions in marine ecosystems (11). In addition to DNA, intra- and interspecies horizontal protein transfer (HPT) has been demonstrated exclusively in vitro to occur through bacterial structures termed “nanotubes” (12). Whether either of these mechanisms for HPT occurs in the gut, by which species,
and how the process is regulated remain unknown. Nonetheless, HPT might emerge as an underappreciated contributor to microbial ecology, with potential implications for virulence and antibiotic persistence and resistance.

In the gut, several pathogens can utilize type 4 and type 6 secretion systems (T4SS and T6SS) to antagonize commensals through translocation of toxins. However, this phenomenon might be broader: Bacteroides spp. can utilize T6SS for intraspecies antagonism, and several Gram-positive bacteria encode a T7SS. In Staphylococcus spp., T7SS was shown to deliver toxins to diverse Gram-positive species (1). Secretion systems might therefore play an important role in shaping the microbial community. The example of T6SS in Bacteroides is of interest, as it confers dominance over a closely related non-T6SS-encoding strain. This trait could potentially be harnessed for microbiome editing by excluding a harmful strain through the introduction of a closely related relative that encodes a dominant trait (T6SS or other) but is engineered to lack the detrimental one. Antagonism of pathogens by gut commensals is also mediated by metabolic products (e.g., short-chain fatty acids and bile acids) and bacteriocins (1). While the latter are considered to have a narrow activity spectrum, our work suggests that some probiotic bacteria have a broad antagonistic impact on the microbiome. Whether this effect is mediated by bacteriocins or other molecules is currently unknown, yet it highlights the importance of identifying factors encoded by probiotics (next generation and current) that can alter the microbiome and determining how these can be manipulated to improve safety and even efficacy.

**FIG 1** Known and putative mechanisms of commensal-commensal interactions in the gut.
An extensively studied interaction in bacterial and fungal communities and biofilms is quorum sensing (QS). In the human gut microbiome, members of *Bacteroidetes* and *Firmicutes* can produce autoinducer-2 (AI-2) molecules. Manipulating AI-2 can facilitate microbiome recovery from antibiotics (13), suggesting further opportunities for beneficial microbiome modulation through QS manipulation. Interestingly, phages also cooperate by chemical sensing of other phages (14). Production of arbitrium peptides by their predecessors allows phage progeny to assess the amount of available bacterial prey and consequently “decide” between lysogeny and lysis. Phages also cooperate to produce a sufficient amount of anti-CRISPR proteins that will immunosuppress their bacterial host. On the other hand, phages can also antagonize other phages by conferring resistance to their bacterial host (14). It remains to be determined if and how these mechanisms of phage interactions manifest in the microbially dense and diverse gut environment. With decades of limited success in applying phage therapy against bacterial pathogens and the growing interest in utilizing phages for specific strain exclusion from the microbiome, a better understanding of these mechanisms would likely be an important component in the efficacy of these therapeutics.

In addition to phages, viral interactions have been demonstrated between human-targeting eukaryotic viruses. Coinfection with two viruses can be beneficial to virus growth and maturation, e.g., adenovirus and adeno-associated viruses; however, it can also result in viral interference, where the presence of one virus alters the infection dynamics of a second virus from a similar or different family. Viral interference can alter the outcome of vaccination (15): live oral but not inactivated polio vaccine (OPV) can prevent picornavirus-associated otitis media; enterovirus infection reduces the efficacy of OPV; and the presence of Sabin poliovirus type 2 in the trivalent OPV interferes with production of a protective antibody response against types 1 and 3, possibly due to competition for receptor binding. It is interesting to speculate whether endogenous production of a protective antibody response against types 1 and 3, possibly due to the gut. Such knowledge will improve our understanding of the formation of dysbiotic communities and how to amend them in an efficient and safe manner. Furthermore, as exemplified by the CRISPR system, discovering new mechanisms of microbial interactions can have far-reaching and unexpected benefits to biomedicine.

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