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Toward the development of defined microbial therapeutics

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Abstract

The collection of micro-organisms living in the mammalian gastrointestinal tract, termed the gut microbiota, has been shown to have profound impacts on host health and increasingly is regarded as a viable therapeutic target. Clinical studies of fecal microbiota transplantation have demonstrated potential efficacy of microbiota-based therapies for diseases including *Clostridioides difficile* infections, inflammatory bowel disease, graft-versus-host disease and cancer. However, the lack of understanding of the active ingredients and potential risks of such therapies pose challenges for clinical application. Meanwhile, efforts are being made to identify effector microbes directly associated with a given phenotype, to establish causality and to devise well-characterized microbial therapeutics for clinical use. Strategies based on defined microbial components will likely enhance the potential of microbiota-targeted therapies.

Keywords: colonization resistance, fecal microbiota transplantation, gastrointestinal infections, gut microbiota, immunomodulation

REVIEW

Introduction

The human body harbors a diverse community of commensal bacteria commonly referred to as the microbiota. Most of the commensals reside in the gastrointestinal tract, which in humans is estimated to contain ~38 trillion bacteria across ~1000 species (1). This gut microbiota community has co-evolved with the host for millennia and provides the host with many benefits, including but not limited to fermentation of dietary fibers, protection against pathogens and education of the immune system (2). Perturbations of the homeostatic microbiota composition (known as dysbiosis) have been shown to be associated with an extensive list of diseases, including inflammatory bowel disease (IBD), allergy, diabetes, obesity, hypertension and cancer (reviewed in (3)).

As a consequence, there is a growing interest in microbiota-based interventions to restore homeostasis and treat diseases. One of the most straightforward ways to manipulate the gut microbiota is through fecal microbiota transplantation (FMT), by which dysbiotic gut microbiota are replaced with healthy microbiota to normalize the composition and hopefully gain therapeutic benefits. This strategy has been demonstrated to be applicable for a number of conditions. Nevertheless, more precise microbiota-based therapeutic strategies are needed in order to maximize the clinical benefits, improve reproducibility and minimize potential adverse effects.

Microbiota research has been revolutionized by a number of recent technological advancements. The advent of high-throughput next-generation sequencing technology has enabled precise and rapid taxonomic characterization of individuals within a complex microbial community (4). Deep metagenome shotgun sequencing (metagenomics), along with other '-omics' approaches such as metabolomics, lipidomics and proteomics, has allowed researchers to perform deeper analysis and reveals new gene products and metabolites with important bioactivities (5). Moreover, gnotobiotic animals, which are born in germ-free conditions and can be colonized with defined microbial communities or strains, provide a valuable tool to identify effector microbiota species responsible for a particular phenotype (6–8). The use of gnotobiotics has been instrumental in shifting microbiota research from association to causality studies. In addition, advancements in culturing technologies have enabled the isolation of many previously uncharacterized species (9).

Recent studies endeavoring to establish causality, especially those using gnotobiotic animal models, have indeed revealed promising effector strains and mechanisms that could be exploited therapeutically. In this review, we discuss recent progress that has been made in the development and application of microbiota therapeutics, focusing on, but not

limited to, infectious and inflammatory diseases. We propose that defined microbial therapeutics, consisting of well-characterized bacteria with validated disease-combating or immunomodulatory properties, hold great promise for microbiota-based interventions.

Progress and challenges in FMT

Clostridioides difficile *infections*

FMT has been shown to be a promising approach to treat recurrent *Clostridioides difficile* infections (CDIs), one of the most significant nosocomial infections (10). Repeated antibiotic exposure, which disturbs the gut microbiota, is a primary risk factor for CDI, as the normal gut microbiota provides colonization resistance against this pathogen (10). Earlier proof-of-concept studies showed striking efficacy of FMT in treating recurrent CDI, with success rates of over 80% and relatively few adverse events (11, 12). Microbiota-based therapeutics containing freeze-dried gut microbiota (e.g. Finch's CP-101) or purified bacterial spores (e.g. Seres's SER-109) derived from healthy donor feces were also demonstrated to be significantly efficacious in reducing CDI recurrence in clinical trials (13). The success of FMT in recurrent CDI provides strong evidence for the causality and effectiveness of microbiota therapeutics and has spurred efforts to test FMT for other indications.

Inflammatory bowel disease

IBD, including Crohn's disease and ulcerative colitis, is another intestinal disorder for which FMT treatment has shown encouraging results. Although the precise pathogenesis of IBD remains elusive, the etiology is likely multifactorial, including genetic susceptibility, dysregulated host immune responses and dysbiosis of the microbiota, with a decrease in biodiversity and a shift in bacterial taxa, including increased abundance of *Enterobacteriaceae* species, such as *Klebsiella pneumoniae* (14, 15). Several clinical trials have been conducted to evaluate the effectiveness of FMT in treating ulcerative colitis and Crohn's disease and the results are varied (reviewed in (16)). In general, FMT showed relatively more encouraging effects in ulcerative colitis, resulting in higher remission rates in patients with active ulcerative colitis or better maintenance of the remission state (16). It is clear that only a fraction of IBD patients responded to FMT treatment, and the determinants (e.g. the specific attributes of the donor/recipient microbiota) that potentially affect the outcome are not well understood.

Gastrointestinal graft-versus-host disease

FMT has been tested in gastrointestinal graft-versus-host disease (GI-GvHD), an anti-host immune response that often occurs following allogeneic hematopoietic cell transplantation (allo-HCT) and leads to negative outcomes. Dysbiosis of the gut microbiota, characterized by loss of diversity and domination by single taxa (e.g. by the *Enterococcus* genus), is known to be associated with GI-GvHD (17, 18). Several studies have been conducted to assess the feasibility and safety of FMT to restore the gut microbiota following allo-HCT (19, 20). The results suggest that FMT has the potential to restore gut microbial diversity and to resolve GI-GvHD, though the small numbers and heterogeneity of the participants in these studies preclude drawing definitive conclusions. The immunocompromised status of allo-HCT recipients warrants extra caution in the use of FMT in this patient group; therefore, more-stringent donor screening protocols are required.

Immune checkpoint inhibitor therapies

There is an emerging interest in using FMT to increase the effectiveness of immune checkpoint inhibitor (ICI) therapies. The microbiota has been shown to affect responses of tumors (melanoma, in particular) to ICI immunotherapy in mouse models and patient cohorts (21, 22). Recently, two proof-of-concept clinical trials provided evidence that FMT derived from patients who benefited from ICI could help overcome the resistance to ICI therapy of patients with metastatic melanoma (23, 24). Further investigations are needed to confirm the efficacy of FMT-ICI combination therapies, to define effector microbes in the transplant and to evaluate to what extent the pioneering success in melanoma can be generalized to other cancer types.

Challenges

Despite the encouraging outcomes of FMT in the abovementioned conditions, there are still a number of challenges associated with its clinical use. One of the biggest issues is the undefined nature of its 'active ingredients'. Therefore, even in diseases in which FMT has demonstrated convincing efficiency (e.g. CDI), there are still regulatory challenges to overcome for its approval as a treatment option. Moreover, as FMT-based therapeutics are derived from donor fecal material, the supply is inherently limited. In addition, fecal material from different donors will have considerable variability, affecting the efficacy and reproducibility of FMT-based therapies. Last but not least, the complex composition of the fecal material also raises concerns over its safety and cases have been reported in which transmission of multidrug-resistant organisms led to fatal consequences (25).

Moving toward defined microbial therapeutics

Comparing with FMT, therapeutics based on defined effector micro-organism(s) are advantageous in many ways. They are easier to manufacture, more consistent between batches and potentially safer. The field is still in its infancy and identifying the microbial candidates with actual therapeutic effects is a formidable task. Nevertheless, accumulating evidence suggests that this is a feasible approach and has the potential to revolutionize microbiota therapeutics. As detailed below, there are various factors to take into consideration when selecting effector strains.

Target pathogens and pathobionts with defined microbial strains

The *Escherichia coli* Nissle 1917 strain, isolated in the pre-antibiotic era from the feces of a German soldier who was resistant to dysentery, is possibly the first defined microbial therapeutic developed. It has been demonstrated to be highly

effective against intestinal colonization by *Enterobacteriaceae* pathogens such as *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) and adherent invasive *E. coli* (AIEC, a pathobiont frequently isolated from Crohn's disease patients) (26, 27). Nissle 1917 produces a breadth of high-affinity iron chelators termed siderophores, which enable it to compete for this essential micronutrient with pathogens (26). Nissle 1917 also produces anti-microbial peptides called microcins which, coupled with siderophores, enter bacteria via the siderophore receptor and target the proton channel of the ATP synthase in the periplasm, leading to inner membrane depolarization and cell death (28).

Defined microbial therapeutics hold promise in the treatment of vancomycin-resistant *Enterococcus* (VRE), a leading cause of hospital-acquired infections (29). In the setting of allo-HCT, antibiotic-mediated depletion of the indigenous microbiota can lead to VRE expansion in the intestine, predisposing the patients to VRE bacteremia (29). It has been shown that a consortium of four bacterial species, consisting of *Bacteroides sartorii*, *Parabacteroides distasonis*, *Clostridium bolteae* and *Blautia producta*, could eliminate VRE colonization in mice (30). In particular, the lantibiotics-producing *B. producta* strain in the consortium was shown to be the major contributor to VRE decolonization (31). Lantibiotics are a class of polycyclic peptides with anti-microbial properties against Gram-positive bacteria through inhibition of cell wall synthesis and formation of pores in the membrane (32). Modulation of dietary nutrients may represent a complementary microbial therapeutic strategy to target VRE infection and colonization, as the growth of *Enterococcus* species was shown to be dependent on lactose (18). A lactose-free diet inhibits *Enterococcus* outgrowth in mice, whereas individuals harboring a lactose-malabsorption allele, resulting in an increase in intestinal luminal lactose, tend to experience extended *Enterococcus* domination after antibiotic treatment (18).

As exemplified by the success of FMT trials, infection by *C. difficile* is another condition that can potentially benefit from defined microbial therapeutics. Under certain environments, such as increases in the availability of monosaccharides and sugar alcohol (33, 34), *C. difficile* germinates from spores and produces TcdA and TcdB toxins. It has been shown that the presence of secondary bile acid-producing bacteria, such as *Clostridium scindens*, is associated with resistance to CDI and that administration of *C. scindens* recovers colonization resistance against *C. difficile* in antibiotic-exposed mice (35). Biosynthesis of secondary bile acids is a multi-step process involving deconjugation, oxidation, epimerization and 7α-dehydroxylation reactions. *Clostridium scindens* is a member of a small fraction of intestinal microbes that possess the complete 7α-dehydroxylation pathway to produce deoxycholic acid (DCA) and lithocholic acid (LCA). DCA and LCA, as well as isoDCA, isoLCA and isoalloLCA have been shown to inhibit *C. difficile* germination, proliferation and/or toxin production (36, 37). Among these bile acids, isoalloLCA has the strongest growth-inhibitory effect and isoalloLCA-producing *Odoribacteriaceae* strains could be exploited to combat CDI (37).

In addition to microbes exerting metabolite-mediated inhibition, those producing bacteriocins that specifically target *C. difficile* could be used. Thuricin CD, a bacteriocin derived from the commensal *Bacillus thuringiensis*, was shown to kill a wide range of clinical *C. difficile* isolates with little effect on commensal members of the microbiota (38). The pathogenesis of CDI as well as infections by other pathogens involves several factors, including toxin-mediated epithelial disruption, activation of the host immune response and changes in the dynamics of the resident microbiota. Indeed, it has been shown that Foxp3$^+$ T-regulatory cells (Tregs) are required for FMT-mediated resolution of CDI in mice (39). To manage such multiple aspects simultaneously, rationally designed complex microbial consortia would be more effective than a single strain (Fig. 1).

Exploit colonization-resistance mechanisms to combat infections

Nutrient competition. As demonstrated by the earlier example of the iron-competing Nissle 1917, competition for limited nutrient resources is a viable approach to target pathobionts. The mature gut microbiota is relatively stable against perturbations, and exogenous species therefore will be forced to compete with the densely populated endogenous species for nutrient niches to colonize. It has been shown that *Citrobacter rodentium*, an attaching and effacing murine pathogen, could be outcompeted by commensal *E. coli* as they share catabolic preferences for monosaccharides (40). Shifting the diet to simple sugars also forced *Bacteroides* species, which otherwise catabolize both monosaccharides and polysaccharides, to compete with C. *rodentium* for monosaccharides (40). S. Typhimurium could be targeted with a similar strategy. This pathogen relies on microbiota-liberated mucosal carbohydrates, such as sialic acid and fucose, for its expansion (33). Antibiotic-mediated disruption of the resident microbiota results in an increase in mucosal carbohydrate availability for S. Typhimurium to expand (33). Conceivably, introducing an ecosystem with endogenous microbiota members that preferentially utilize free sialic acid and fucose could help restrict *Salmonella* expansion. Intestinal nutrient competition goes beyond metal and carbon sources. For example, butyrate-producing *Clostridia* can reduce oxygen availability in the colon by driving the energy metabolism of colonocytes toward β-oxidation in the mitochondria, thereby inhibiting the growth of facultative anaerobic enteric pathogens such as S. Typhimurium (41). Commensal *Enterobacteriaceae* can further reinforce colonization resistance against *Salmonella* by consuming the small amount of oxygen emanating from the epithelial surface (42).

Active antagonism. Members of the microbiota can actively antagonize other species through production of bacteriocins, type VI secretion system (T6SS)-dependent antibacterial effectors and bacteriophages. Bacteriocins are a heterogeneous group of proteinaceous toxins secreted by bacteria. These include small anti-microbial peptides such as microcins, lantibiotics and thuricin CD discussed earlier, as well as large proteins such as colicins produced by E. coli. Colicins target related *Enterobacteriaceae* species possessing specific receptors but lacking cognate immunity

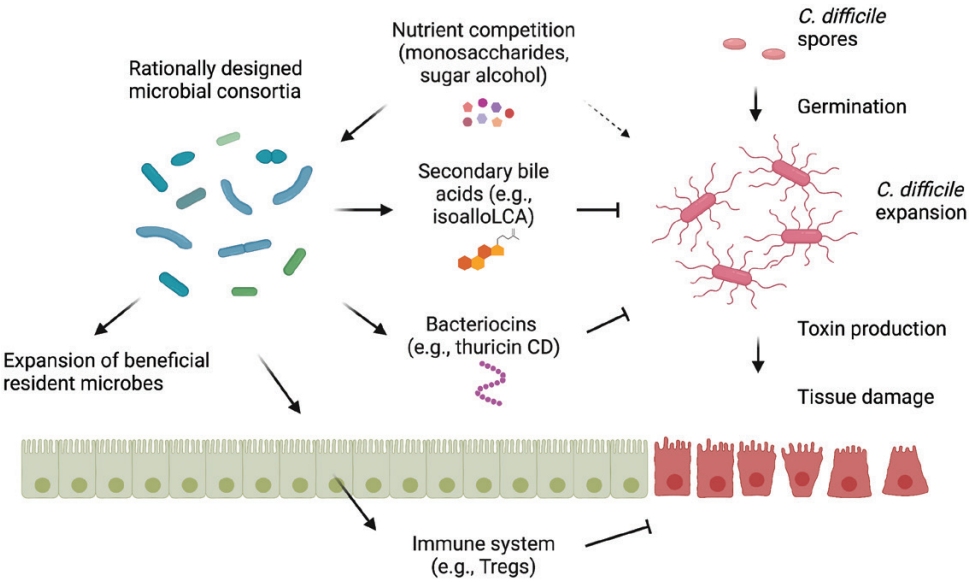

Fig. 1. Design of rationally defined microbial consortia for CDI. A rationally designed microbial consortium including bacterial strains that compete for nutrients (monosaccharides in particular), produce secondary bile acids and bacteriocins, nurse resident beneficial microbes and activate the host immune system (Treg cells in particular) could be effective to suppress *Clostridioides difficile* germination from spores, expansion, production of toxins, induction of epithelial damage and inflammation.

proteins (43). Dietary inclusion of a colicin has been shown to prevent post-weaning diarrhea caused by enterotoxigenic *E. coli* in piglets (44). Another mechanism of bacterial antagonism is through T6SS-dependent, cell-to-cell contact delivery of effector molecules. T6SS transports peptides or proteins (many of them are toxic effectors) across the inner and outer membrane of Gram-negative bacteria and into neighboring related strains (45). For instance, colonization with a T6SS-expressing commensal *Bacteroides fragilis* confers resistance to enterotoxigenic *B. fragilis* (ETBF) in mice (46). Bacteriophages, which have high host-specificity and typically infect and kill a single species, can similarly contribute to interspecies competition and have the potential to be exploited as a therapeutic tool. For instance, a strain of *Enterococcus faecalis* was reported to produce phages to outcompete other *E. faecalis* strains (47).

Manipulate host immunity with defined immunomodulatory microbiota members

The microbiota has been demonstrated to contribute to almost every aspect of the mucosal immunity in the intestine. The presence of the microbiota contributes to the maintenance of barrier integrity of intestinal epithelial cells, enhances mucus production by goblet cells, promotes the secretion of antimicrobial peptides by Paneth cells and IgA by plasma cells to restrict bacterial expansion and penetration and stimulates the activation and differentiation of different subsets of intestinal T cells as well as other immune cell populations (extensively reviewed in (48)).

A number of studies have provided evidence that defined microbiota members can be used as therapeutics to promote the development of specific groups of immune cells. For instance, a human commensal *B. fragilis* was shown to drive the differentiation of IL-10-expressing Tregs and confer protection against different models of experimental colitis in a polysaccharide A (PSA)-dependent manner (49). Similarly, a rationally selected mixture of 17 *Clostridia* strains isolated from a human microbiota was shown to induce colonic Treg differentiation (7). The immunomodulation is at least in part due to the action of *Clostridia*-derived short chain fatty acids (SCFAs), such as butyrate (50).

Endogenous microbiota members have been shown to induce other subpopulations of T cells. For example, segmented filamentous bacteria (SFB) (6) and *Bifidobacterium adolescentis* (51) were identified to specifically induce T helper 17 (Th17) cells in the intestine. The Th17-inducing property of SFB was demonstrated to be dependent on their direct association with the gut epithelium (52). SFB stimulate microbial adhesion-triggered endocytosis in epithelial cells and induce SFB-antigen-specific, tissue-resident and homeostatic Th17 cells (53). The homeostatic Th17 cells contribute to the maintenance of epithelial barrier integrity, which affords protection against pathogens such as *C. rodentium* (6). However, Th17 responses are also implicated in IBD and other autoimmune disorders (54). The pro-inflammatory Th17 cells are shown to be induced by IL-23 and serum amyloid A proteins as well as by a glycolysis-promoting hypoxic environment (55, 56). Therefore, by leveraging the understanding of induction programs of homeostatic versus pro-inflammatory Th17 cells, Th17-inducing microbiota could be exploited to boost host anti-microbial responses.

Besides Treg-inducing and Th17-inducing bacteria, a consortium of 11 bacterial strains that can robustly induce interferon-γ-producing CD8 T cells in the intestine was recently isolated from healthy human donor feces (8). Administration of the 11 strains conferred resistance to *Listeria monocytogenes* and enhanced ICI-mediated anti-tumor immunity in mice (8). Clinical trials are underway to

evaluate the 11 strains as a potential treatment option for patients with advanced or metastatic cancers (13).

Implications and perspectives

Microbiota-based therapeutics have the potential to become a major therapeutic modality, which encompasses those targeting specific pathogens/pathobionts as well as those aiming to rationally restructure a given microbial community in a specific way. Although in this review we have focused our discussion on therapeutics comprising live bacteria, there are other microbial therapeutic strategies under development. The alternative strategies include those involving administration of metabolites derived from microbial sources and those based on non-bacterial micro-organisms such as bacteriophages. Metabolite- and bacteriophage-based therapeutics may have more targeted effects and overcome some of the potential issues associated with live bacterial therapeutics, such as engraftment and inter-individual variations. However, as the mechanisms underlying the pathogenesis of many microbiota-related diseases are usually manifold, strategies targeting a single pathway or entity may not be sufficient. Bacterial communities, on the other hand, can generate interactive networks to cooperatively produce a large variety of bioactive molecules and act through multiple mechanisms. Therefore, live bacterial consortia composed of multiple species have the potential to elicit more effective and durable clinical responses than the alternatives. Nevertheless, complementary strategies should be considered to optimize the design of live bacterial consortia, for instance, by including genetically modified bacteria that secrete desired metabolites, proteins or bacteriophages to target specific pathways or microbes. Inclusion of prebiotics should also be considered to improve the engraftment of effector strains. Employing multiple strategies simultaneously will likely maximize the benefit of future microbiota-targeted therapeutics.

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