The Journal of Immunology

REVIEW ARTICLE | JANUARY 15 2024

Engineering Strategies to Modulate the Gut Microbiome and Immune System

Kai Han; ... et. al

J Immunol (2024) 212 (2): 208–215.
https://doi.org/10.4049/jimmunol.2300480

Brief Reviews

The Journal of Immunology

Engineering Strategies to Modulate the Gut Microbiome and Immune System

Kai Han,[*,†,‡,1] Jin Xu,[*,‡,1] Fang Xie,[*,‡,1] Julia Crowther,[*,‡] and James J. Moon[*,‡,§,¶]

The gut microbiota, predominantly residing in the colon, is a complex ecosystem with a pivotal role in the host immune system. Dysbiosis of the gut microbiota has been associated with various diseases, and there is an urgent need to develop new therapeutics that target the microbiome and restore immune functions. This *Brief Review* discusses emerging therapeutic strategies that focus on oral delivery systems for modulating the gut microbiome. These strategies include genetic engineering of probiotics, probiotic-biomaterial hybrids, dietary fibers, and oral delivery systems for microbial metabolites, antimicrobial peptides, RNA, and antibiotics. Engineered oral formulations have demonstrated promising outcomes in reshaping the gut microbiome and influencing immune responses in preclinical studies. By leveraging these approaches, the interplay between the gut microbiota and the immune system can be harnessed for the development of novel therapeutics against cancer, autoimmune disorders, and allergies. *The Journal of Immunology*, **2024, 212: 208–215.**

The gut microbiota is a complex and fascinating ecosystem in which gut microbes are predominantly localized in the colon (10^{12} microbes/g), with a lesser amount located in the small intestine (10^{8} microbes/g) (1). Microbial colonization of the gastrointestinal (GI) tract begins as early as birth and takes several years to mature into a relatively stable gut microbial community (2). Although the composition of gut microbiota changes markedly with age in infants, they still display a beneficial influence on infants' immune systems (e.g., the establishment of tolerance to allergens) (3). Microbes interact locally with the intestinal immune system, where mucins secreted by goblet and epithelial cells act as physical and biochemical barriers, preventing microbes from entering the systemic circulation. More specifically, dense mucins in the colon can prevent the development of chronic inflammatory bowel diseases (IBDs) (4), while loose mucins in the small intestine can coat Ags derived from microbes and deliver them to dendritic cells (DCs) to induce oral tolerance (5). Epithelial cells can generate antimicrobial peptides, which act as natural antibiotics to kill or inactivate microbes (6), or they can release anti-inflammatory cytokines (e.g., IL-10, TGF-β) in response to microbial-derived signals (7, 8). Besides, microbes can manipulate host immunity systemically. Microbes rely on dietary components, such as carbohydrates and proteins, for nutrition. Microbial degradation of dietary components leads to the generation of immunomodulatory amino acids and microbial metabolites, including short-chain fatty acids (SCFAs) (9). Microbes and their endotoxins can migrate directly through the intestinal barrier, thus potentially modulating various pathologies, such as sepsis (10) and cancer (11, 12).

Increasing evidence has shown that modulation of the composition and diversity of the gut microbiome can reshape the immune system in pathological conditions. For instance, oral administration of *Akkermansia* or *Bifidobacterium pseudolongum* can improve the therapeutic outcomes of immune checkpoint blockers (ICBs) (13–15), and fecal microbiota transplant (FMT) can accelerate the clearance of pathogens in sepsis by normalizing the host immunity (16). Current strategies for gut microbiota modulation mainly include live therapeutic products (e.g., FMT, probiotics, and bacteriophages) and bioactive compounds (e.g., prebiotics, antibiotics, and microbial metabolites). Among them, FMT has been widely investigated with >200 clinical trials registered to date (17), most notably for the treatment of *Clostridium difficile* infection. FMT is thought to replenish the microbial diversity lost after antibiotic treatment, restore microbial metabolites, and protect against *C. difficile* colonization. However, FMT poses potential risks, including safety issues associated with pathogenic microbes, and FMT is a challenging drug product to produce on a large scale and regulate.

Modern engineering strategies can optimize the biodistribution and pharmacokinetics of chemical drugs and biomolecules in vivo and have achieved great success in various therapeutics. However, engineering strategies for the gut microbiome and immune system are still in the infant stage. In this *Brief Review*, we discuss the

*Department of Pharmaceutical Sciences, University of Michigan, Ann Arbor, MI; †State Key Laboratory of Natural Medicines, Department of Pharmaceutics, China Pharmaceutical University, Nanjing, China; ‡Biointerfaces Institute, University of Michigan, Ann Arbor, MI; §Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI; and ¶Department of Chemical Engineering, University of Michigan, Ann Arbor, MI

[1]K.H., J.X., and F.X. contributed equally to this work.

ORCIDs: 0000-0001-5370-4296 (K.H.); 0009-0009-7357-2046 (J.C.); 0000-0003-2238-2372 (J.J.M.).

Received for publication July 17, 2023. Accepted for publication August 28, 2023.

This work was supported in part by National Institute of Dental and Craniofacial Research Grants R01DE030691 and R01DE031951, Division of Diabetes, Endocrinology, and Metabolic Diseases Grant R01DK125087, National Cancer Institute Grant R01CA271799, and by National Institute of Neurological Disorders and Stroke Grant R01NS122536.

Address correspondence and reprint requests to Prof. Kai Han and Prof. James J. Moon, University of Michigan, Department of Pharmaceutical Sciences, Ann Arbor, MI 48109. E-mail addresses: kaihan@cpu.edu.cn (K.H.) and moonjj@umich.edu (J.J.M.)

Abbreviations used in this article: AHR, aryl hydrocarbon receptor; AM, amphiphilic molecule; DC, dendritic cell; eATP, extracellular ATP; EcN, *Escherichia coli* Nissle 1917; FMT, fecal microbiota transplant; GELN, ginger-derived exosome-like nanoparticle; GI, gastrointestinal; HA, hyaluronic acid; HABN, HA–bilirubin conjugate self-assembled into a nanostructure; IBD, inflammatory bowel disease; ICB, immune checkpoint blocker; LDL, low-density lipoprotein; OMV, outer membrane vesicle; ROS, reactive oxygen species; Treg, regulatory T cell.

https://doi.org/10.4049/jimmunol.2300480

recent engineering strategies for modulation of the gut microbiome and regulation of the immune responses for treatments against various pathologies, including cancer, autoimmune disorders, and allergy diseases (Fig. 1). We focus on oral formulations due to their potential direct interactions with the gut microbes in the intestine, widespread clinical applications, and high patient compliance.

Engineering oral formulations for gut microbiota and immune modulation

Direct genetic engineering of probiotics. Reconstitution of the gut microbiome with a defined and specific microbial population holds great promise to modulate the intestinal microenvironment and host physiology in a safe and precise manner and thus provides an attractive alternative strategy to FMT. Synthetic biology is another approach that can therapeutically manipulate microbial cells. For instance, the probiotic strain *Escherichia coli* Nissle 1917 (EcN) is an advanced toolbox for its genetic manipulation and has a long history of safe uses in humans (18). Genetically engineered strains of the probiotic have entered clinical trials for a variety of functions, including local metabolic modulation in the intestine for the treatment of hyperammonemia and phenylketonuria, and intratumorally for stimulator of IFN genes (STING) activation (19–21). Moreover, recent advances in gene circuit design principles have enabled on-demand designs, capable of sensing disease-associated metabolites and regulating their degradation or function in the gut (22). For example, Koh et al. (23) engineered EcN for inhibiting *C. difficile* infection by restoring bile acid metabolism in response to intestinal microbiome dysbiosis. They used pNanA as a genetic sensor for sialic acid, a marker for microbiome dysbiosis, to regulate the expression of the bile salt hydrolase Cbh. The authors showed that the deconjugated and more active form of Cbh, taurocholate, effectively inhibited both endospore germination and growth of *C. difficile* in vitro and diminished the symptoms of *C. difficile* infection in a mouse model.

In another study, Zou et al. (24) designed an EcN engineered to secrete an immunomodulatory protein AvCystatin in response to thiosulfate, a biomarker for inflammation. The orally administered bacteria ameliorated disease activity in a murine colitis model and significantly decreased immune infiltrates and proinflammatory cytokines, such as IL-6 and IFN-γ. In another study, Scott et al. (25) engineered *Saccharomyces cerevisiae* yeast to sense extracellular ATP (eATP) and secrete CD39-like apyrase, which degrades eATP into immunosuppressive adenosine. The open-loop design where the yeast constitutively secreted apyrase led to unregulated accumulation of adenosine, which triggered fibrosis and tissue damage. In contrast, the closed-loop design used the directed evolution of the human P2Y2 receptor to develop an eATP biosensor that promoted regulatory T cells (Tregs) and provided protection in murine models of chemical-induced colitis and anti-CD3–induced enteritis. These results highlight the capabilities of controlled adaptive systems to balance and regulate the gut inflammatory state.

Another genetic engineering approach involves the utilization of genetic modules for biomaterial or biomolecular assemblies, such as vaccine constructs or cytokine delivery systems. For instance, Yue et al. (26) fused a tumor Ag and an IgG Fc fragment to ClyA, a surface protein found on the outer membrane vesicles (OMVs) of *E. coli* strain Top10 (termed OMV-Ag-mFc). To increase the expression of the OMV-Ag-mFc construct, an arabinose-inducible promoter was introduced to the assembly. Upon oral administration, the bacteria produced OMV-Ag-mFc, which penetrated the intestinal epithelial barrier and was taken up by DCs in the lamina propria via FcRn. With Adpgk used as a model Ag, the engineered *E. coli* elicited a stronger CD8 T cell response and inhibited MC38 tumor growth. In another study, Chen et al. (27) reported a topical vaccine, containing the commensal bacteria *Staphylococcus epidermidis,* to generate tumor-specific T cells that can migrate to local and metastatic tumor lesions where they exert cytotoxic functions. Praveschotinunt et al. (28)

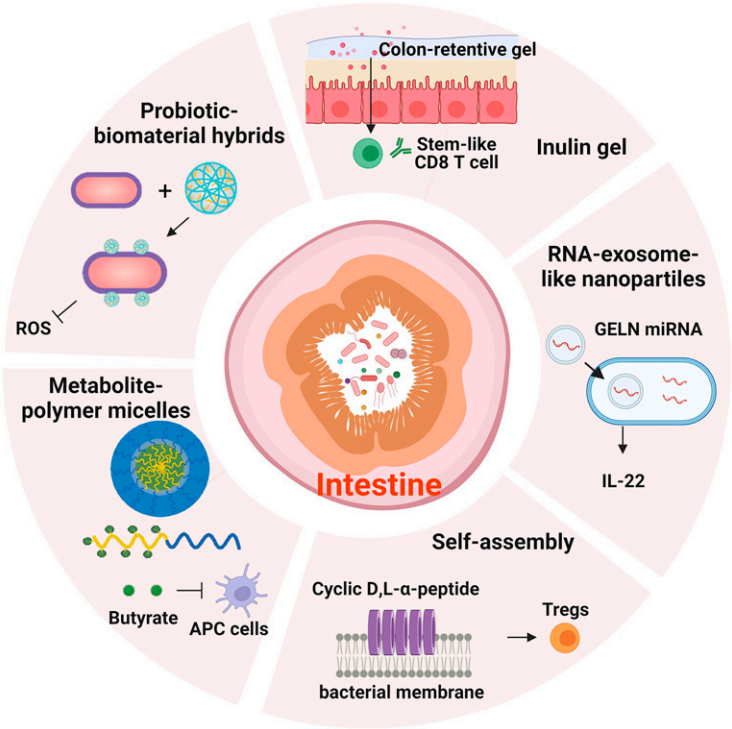

FIGURE 1. Recent advances in engineered oral formulations for modulating the gut microbiome and immune system. Various oral formulations, including probiotic/biomaterial hybrids (100), metabolite/polymer micelles (72), self-assembled peptides (90), RNA delivery nanoparticles (94), and dietary fiber gel (49), have been developed to restore the intestinal barrier, microbial community structure, and microbial-derived metabolites. These approaches can influence the activation or differentiation of APCs, $CD4^+$ T cells, or $CD8^+$ T cells, redirect the secretion of inflammatory or anti-inflammatory cytokines, and exert therapeutic efficacy in various pathologies. This figure was created with BioRender.com. Some images used in this figure were taken from figures in Refs. 72 and 100 under a CC-BY-NC license.

designed a "probiotic-associated therapeutic curli hybrids (PATCH)," where they programmed EcN to secrete CsgA proteins, also known as curli fibers, fused to trefoil factor family peptides, and PATCH was able to promote cytokine secretion as well as epithelial healing. More specifically, these proteins formed extracellular fiber matrices that multivalently displayed the trefoil factor family domains, leading to mucosal healing and attenuation of colonic inflammation in dextran sulfate sodium colitis models.

Probiotic/biomaterial hybrids. Direct oral administration of probiotics could significantly diminish their therapeutic efficacy due to the harsh environmental conditions along the GI tract. In this regard, protecting probiotics with an environment-responsive coating with biomaterials holds great promise in preventing inoculant inactivation and promoting stable engraftment (29, 30). Moreover, the combination of biomaterials and genetic engineering has shown great potential in improving probiotic delivery. For example, Yang et al. (31) coated EcN with tannic acid, Ca^{2+}, and mucin via layer-by-layer technology (termed EcN@TA-Ca^{2+}@Mucin) (31). In this design, tannic acids functioned both as a reactive oxygen species (ROS) scavenger and anchor for mucin, thus enhancing intestine adhesiveness. The authors demonstrated an enhanced colonization of EcN@TA-Ca^{2+}@Mucin along the intestinal tract when compared with EcN alone or EcN@TA-Ca^{2+} without mucin. EcN@TA-Ca^{2+}@Mucin inhibited inflammation, repaired the mucus layer in a dextran sulfate sodium colitis model, and increased the relative abundance of the *Lachnospiraceae* NK4A136 group and *Bifidobacterium adolescentis*. In another study, Zhou et al. (32) coated EcN with chitosan and sodium alginate via layer-by-layer electrostatic self-assembly. EcN was genetically engineered to overexpress catalase and superoxide dismutase to eliminate ROS. Chitosan/alginate-coated EcN markedly inhibited inflammation and restored the intestinal barrier function in multiple chemical-induced IBD models when compared with uncoated EcN. Coated EcN also regulated the intestinal microbiome and increased the relative abundance of *Lachnospiraceae* NK4A136 and *Odoribacter*, which are known butyrate-producing bacteria with anti-inflammatory capabilities (33). Nanoparticles have also been used to form probiotic/biomaterial hybrids. For instance, Pan et al. (34) reported a polyphenol-based nanocoating system referred to as "nanoarmor" composed of tannic acids and ferric ions. In this system, probiotics individually coated with the supramolecular network were filled into enteric capsules for oral delivery. Nanoarmor adsorbed antibiotics, promoted probiotic populations in the GI tract of levofloxacin-treated rats, and reduced the incidence of antibiotic-associated diarrhea. In another study, Liu et al. (35) encapsulated EcN with norepinephrine, which formed a polymer coating via auto-oxidation and promoted mucosa adhesion through its catecholamine group. The authors further engineered EcN into self-assembled nanoparticles by conjugating poly(propylene sulfide), a known ROS scavenger, to hyaluronic acid (HA). This hybrid nanoparticle was able to reduce the number of immune infiltrates and improve therapeutic efficacy in a mouse model of colitis, likely by increasing the relative abundance of beneficial commensals, such as *Muribaculaceae* and *Prevotellaceae UCG-001*, while decreasing pathogenic *Desulfovibrionaceae*. EcN OMVs also have been shown to exert anti-inflammatory effects in experimental colitis models (36, 37).

Engineering dietary fibers into oral formulations

Dietary fibers have an extensive effect on the structure of the gut microbial community throughout one's lifespan. Gut microbes can ferment certain dietary fibers to produce metabolites such as SCFAs (38) or improve glucose and lipid metabolisms (39, 40). These fermentation products can in turn manipulate the host immunity and gut microbial composition. For example, melanoma patients consuming a high-fiber diet have been reported to have a higher abundance of fiber-fermenting *Ruminococcaceae* with a higher response rate to ICB therapy (41). Li et al. (42) further demonstrated that the low-fiber diet promoted resistance to ICBs in a murine model of LSL-$Kras^{G12D}$ lung cancer. A potential mechanism for this increased antitumor efficacy associated with a high-fiber diet is the production of type I IFN by intratumoral monocytes in response to microbiota-derived STING agonists (43). Beyond the consumed dose of dietary fibers, interactions between the gut microbes and dietary fibers are also affected by the chemical structure of dietary fibers as well as their degree of polymerization, solubility, and exposure to high temperatures (44–46). Therefore, engineered dietary fibers can address unique nutrient niches in the intestine that favor specific microbes, promoting their proliferation and colonization (47).

Current research focuses on investigating the interactions between dietary fibers and gut microbiota in animal models by adding native fibers to chow. A potential strategy for dietary fiber engineering is to develop a hydrogel, similar to those that have been applied in typical cooking methods, such as potato starch gel and kudzu powder gel. These hydrogels are usually prepared via a simple "heating-cooling" treatment and show higher viscosity compared with native dietary fibers (48), thus increasing their transit time through the intestines. In our previous work, we performed an in vivo screening of various dietary fibers, polyphenols, and drugs from the U.S. Food and Drug Administration's generally recognized as safe list and found that inulin, a polysaccharide dietary fiber, synergized with ICBs by increasing the relative abundances of favorable bacteria, such as *Akkermansia*, *Lactobacillus*, and *Roseburia* in the colon (49). Based on this, we have developed a viscous, oral inulin gel formulation, which prolonged its colonic retention compared with the native inulin. As most of the body's gut microbes reside in the colon, inulin gel increased the relative abundance of these beneficial microbes, which released SCFAs that induced the formation of stem-like memory T cell factor-1^+PD-$1^+$$CD8^+$ T cells in both tumor and tumor-draining lymph nodes, thereby improving the therapeutic outcome of ICBs (49). This hydrogel engineering strategy may not only improve the biological functions of dietary fibers but also serve as stabilizers or carriers to deliver immunomodulatory drugs and biomolecules to specific sites in the intestines.

Engineering endogenous biomolecules into oral formulations

Microbial metabolite delivery system. Modulating the gut microbiome compositions through genetic engineering techniques is straightforward and highly specific (50). Nevertheless, the intricacy of the gut microbiome, metabolomics, and interactions with host immunity poses numerous hurdles, including safety concerns and regulation considerations (51–53). Alternatively, prebiotics offer safe and effective means of shaping the gut microbiota composition; however, prebiotic-based approaches can be limited due to

maximum tolerated doses and influenced by environmental factors while lacking selectivity for enriching bacteria of interest (54–56).

Dysbiosis of gut flora and changes in microbial metabolites are often associated with immune dysregulations, and vice versa (57–59). Although numerous molecules are produced by bacteria in the GI tract, the mechanism and complexity of their interactions remain elusive. Microbial metabolites such as SCFAs, specifically butyrate, have been studied for their effects on the human immune system. These include maintaining epithelium barrier integrity, inhibiting histone deacetylase activities, and modulating cell differentiation and function (60–63). Reduced abundance of butyrogenic bacteria has been reported in various immunological diseases such as allergies, colitis, type 1 diabetes, multiple sclerosis, and cancer (3, 64, 65). Therefore, engineering SCFAs for gut microbiome and immune modulation presents a pragmatic strategy.

Many efforts have been made to deliver SCFAs orally. Previous studies explored different routes of administering acetate in mice and humans (66, 67). Oral gavage and i.p. administration were found to significantly increase plasma acetate levels but only transiently, with rapid clearance within an hour. Many clinical trials involving SCFAs have used sodium salts or triglycerides (68–70), which are quickly absorbed, metabolized, and thus unable to reach the lower GI tract. Even with enteric coating or encapsulation, SCFAs give off strong odors and have an unpalatable taste (71), likely discouraging patient compliance. Therefore, feasible and controlled approaches are needed for engineering and delivering SCFAs to the distal GI tract, where they can interact with colon-residing commensal microbes and host immune cells.

Wang et al. (72) developed amphipathic copolymers that are capable of self-assembling into nanomicelles in aqueous solutions. Butyrate was conjugated to the hydrophobic block of the copolymer via ester bonds and linked to the hydrophilic blocks with either neutral or negatively charged polymers. This design ensured that the nanomicelles were resistant to degradation by the acidic stomach environment and burst release of their cargo. Once exposed to esterase in the lower GI tract, the conjugated butyrate was released. However, the difference in surface charges led to distinct release profiles. The neutral micelles exhibited faster release in simulated gastric fluids within a 20-d time frame, and even faster release in the ileum of mice, beginning as early as 2 h after a single oral dose. In contrast, the negatively charged micelles extended the primary release to the cecum region, lasting 8 h compared with 2 h, possibly due to differences in their corona. Subsequently, the combination of these two micelles preserved intestinal barrier integrity, reduced severity of colitis, and protected mice against severe anaphylactic responses in a peanut allergy model. Two weeks of butyrate micelle treatment altered the fecal microbiota composition compared with PBS-treated controls and significantly restored the abundance of *Clostridium* cluster XIVa, a major butyrate-producing taxon associated with beneficial clinical outcomes in patients with Crohn's disease and *C. difficile* infection (73, 74). Further investigation into the immune response showed suppressed myeloid cell activation in the spleens and tissue-draining lymph nodes of mice treated with butyrate micelles, including downregulated expressions of MHC class II and CD86 on DCs and macrophages. The therapeutic potential of these butyrate micelles was further confirmed in the $CD45RB^{hi}$ T cell transfer model of colitis. Two weeks of twice-daily treatment with butyrate micelles successfully alleviated weight loss, preserved colon length, and improved histopathological conditions. These results indicate that engineering butyrate into polymeric micelles allows for direct and non–Ag-specific colonic delivery of SCFAs with a high dosing capacity and minimal adverse effects.

Other early-stage attempts at SCFA delivery include ultrasound-triggered sustained release of SCFAs from a cellulose hydrogel, which was formulated based on the phase inversion method (65, 75). The Morrison and Frost groups conducted clinical trials using SCFAs conjugated to inulin, primarily propionate (76–80), addressing the issues of unpalatability while achieving controlled release of SCFAs and reducing the required amount of the prebiotic fiber. In a recent study, oral dosing of propionate conjugated to inulin decreased proinflammatory IL-8 levels and reshaped the gut bacterial populations at the class and order levels compared with cellulose (81).

Endogenous bioactive molecules. In addition to microbial-derived metabolites, host-generated endogenous bioactive molecules engineered for oral administration can exert immune-modulatory activities and impact the gut homeostasis. Targeted delivery of these molecules to the small intestine or colon poses challenges, such as surviving harsh physiological conditions, minimizing absorption by off-target gastric compartments, enabling site-specific cargo release, and enhancing its bioactivity either with adjuvants or by prolonging its retention in the GI tract.

HA, a glycosaminoglycan biopolymer found in the extracellular matrix and synovial fluids, possesses multiple immunomodulatory properties (82, 83). To overcome challenges associated with HA's susceptibility to gastric acid, enzymes, and oxidative conditions in inflamed tissues within the GI tract, we have previously reported the development of a nanomedicine composed of HA conjugated with bilirubin (BR) (84), another endogenous biomolecule commonly found in bile with high hydrophobicity. The HA–bilirubin conjugate self-assembled into a nanostructure (HABN) in an aqueous buffer, improving resistance to hyaluronidase degradation and efficient colonic targeting to inflamed epithelium and macrophages through HA-CD44 recognition. This platform efficiently targeted inflamed epithelium and macrophages in a murine model of colitis. HABN demonstrated superior therapeutic efficacy compared with conventional IBD treatments by promoting anti-inflammatory immune profiles, restoring epithelial barrier integrity, and improving microbial richness and diversity. Mice receiving HABN treatment had reduced IL-1β, TNF-α, and IL-6 and elevated IL-10 and TGF-β in the lamina propria, decreased frequencies of proinflammatory monocytes and neutrophils, and increased anti-inflammatory tissue-resident macrophages, Tregs, and DCs. The treatment also significantly enriched the relative abundance of *Akkermansia muciniphila*, *Clostridium* XIVα, and *Lactobacillus*, which are associated with increased butyrate production and benefits of gut homeostasis (74, 85, 86).

HA serves as a scaffold when designing platforms for different endogenous molecules. For instance, HA was incorporated in a supramolecular drug delivery system using cucurbituril chemistry (87), where the mesoporous silica core loaded with hydrocortisone was coated with tryptophan-modified chitosan and azobenzene-modified HA linked by cucurbit[8]uril. This platform demonstrated targeted cargo release and activation of the aryl hydrocarbon receptor (AHR) in response to azoreductase and tryptophan derivatives generated by gut commensal microbes, promoting anti-inflammatory responses. Similarly,

HA was used to formulate an oral hydrogel, which was then coated with calcium alginate by an electrospinning technique, for delivering selenium to induce in situ synthesis of selenoproteins in colitis-affected tissues, resulting in suppressed gut inflammation, improved tissue repair, and altered gut microbiota compositions (88).

These studies highlight the versatility of engineering strategies for colon-targeted delivery of endogenous bioactive molecules.

Engineering exogenous molecules into oral formulations

The biomolecules secreted by the host and gut microbes are not limited to the aforementioned endogenous biomolecules but also include RNA and antimicrobial peptides, which can control the growth of gut microbes and exert immunomodulatory functions. Currently, the structure and function of these endogenous biomolecules in the context of various diseases are largely unknown, which restricts their applications in oral therapeutics. Additionally, RNA and antimicrobial peptides can be degraded in the harsh intestinal environment. Thus, engineered formulations for delivering these biomolecules could improve their oral bioavailability in the GI tract and control their release behavior at the targeted site, thereby maintaining their biological functions in the gut microbiota and immune system.

Oral formulation of antimicrobial peptide self-assembly. Cationic α-helical antimicrobial peptides have been widely investigated due to their ability to lyse bacterial membranes, but their clinical translation is impeded due to their proteolytic degradation-mediated structural instability and in vivo hemolysis toxicity (89). To address these limitations, Chen et al. (90) developed an in vitro en masse screening approach and a distance scoring algorithm, which identified that cyclic D,L-α-peptides selectively partitioned into bacterial membranes. These cyclic peptides are self-assembled into extended β-sheet–like hollow tubular structures with improved stability due to their even number of alternating D- and L-α-amino acid configurations. Daily oral administration of these cyclic peptides remodeled the gut microbiome in Western diet–fed low-density lipoprotein (LDL) receptor–null mice toward a healthy status. Mechanistically, these peptides mediated metabolic changes and transcriptional reprogramming of the gut microbiota. More specifically, they increased butyrate metabolism and ileal expression of cholesterol 7-α-hydroxylase (known as a rate-limiting enzyme to convert dietary cholesterol to bile acids), leading to reduced plasma LDL cholesterol levels. Notably, the cyclic peptide treatment reduced the absolute number of Th17 cells and Th17-like Tregs but maintained the population of regenerative Helios$^+$ thymic-derived Tregs expressing GATA3 in LDL receptor–null mice with a Western diet, highlighting their potential applicability in other autoimmune and inflammatory diseases (90).

Oral formulation for RNA delivery. There is accumulating evidence that RNAs shape the gut microbiome (91). More specifically, some RNAs generated from gut epithelial cells and Hopx$^+$ cells can enter microbes and facilitate host control of the gut microbiota (92). RNAs can be packaged into gut bacteria-derived extracellular vesicles to affect host pathways (93). Teng et al. (94) engineered ginger-derived exosome-like nanoparticles (GELNs) containing RNAs that can be directly taken up by gut microbes to alter the microbiome community structure and host physiology. Interestingly, the lipid composition of ELNs could influence their localization in both tissues and microbes. A higher content of phosphatidic acid in ELNs improved their accumulation in the gut and accelerated uptake in *Lactobacillus rhamnosus*. However, a higher content of phosphatidylcholine lipid promoted the migration of ELNs from the intestine to the liver. Phosphatidic acid–enriched GELN RNAs increased the relative abundance of *Lactobacillaceae*. Specifically, GELN-derived mdo-miR7267-3p has a binding site for mRNA encoding *Lactobacillus rhamnosus* monooxygenase ycnE, which has been shown to inhibit the colonic expression of the enzyme ycnE, as well as the conversion of tryptophan to indole-3-acetamide. In return, GELN-RNAs favored the release of metabolites including indole-3-carboxaldehyde, which is known as a ligand for AHR. This metabolite promoted the expression of colonic IL-22 via the AHR pathway, improved the gut barrier function, and protected against colitis in mice.

Oral formulation for controlled release of antibiotics. Antibiotics are another class of molecules that can dramatically change the gut microbiome. Although antibiotics are frequently used to protect against pathogenic infections, oral antibiotics are not able to selectively eliminate pathogens, leading to the disruption of gut microbial diversity and a decrease in the host immune response to therapeutic Abs and drugs, such as ICBs (13–15). Oral antibiotics also contribute to the emergence of resistant bacterial strains through repeated, unnecessary, and excessive administration of antibiotics (95). However, some patients cannot avoid antibiotic regimens due to long-term therapies compromising their immune systems. Current engineering strategies for oral antibiotic delivery primarily focus on minimizing the adverse effects of antibiotics to maintain homeostasis of the gut microbiome and host immunity. One strategy delivers adsorbent to the ileum and neutralizes antibiotics in the proximal colon (96). This is achieved through formulating an enteric-coated activated, charcoal-based product, DAV132. Neutralization of antibiotics in the colon aids in preserving beneficial commensal organisms, and targeted delivery of the adsorbent to the ileum allows for more precise targeting of pathogenic bacteria (96). Another strategy focuses on the various receptors in the small intestine that are essential for the absorption of nucleosides, bile acids, and glucose (97). These receptors can serve as a target for enhanced delivery of oral antibiotics through the small intestine and can potentially mitigate their detrimental effects on colonic microbes. Zhang et al. (98) designed positively charged, glucosylated nanoparticles that facilitate the transfer of antibiotics from the proximal small intestine (high expression of the sodium-dependent glucose transporter-1) into the bloodstream via glucose–glucose–transporter binding interactions. This engineering strategy markedly decreased the adverse effects on intestinal microbiota and reduced inflammatory cytokines and neutrophils in the lungs.

The pathological intestinal microenvironment can also be used as a target for infection-mediated release of antibiotics. Mu et al. (99) engineered a H_2S-cleavable amphiphilic molecule (AM) composed of xylooligosaccharide analogs conjugated with 1-dodecanethiol through a disulfide bond and packaged into AM vesicles for antibiotic delivery. In salmonellosis, the pathogen *Salmonella* could produce H_2S, which disrupts the AM vesicles and induces a controlled release of antibiotics. This engineering strategy prevented significant changes in the gut microbiota, achieved localized targeting of the pathogen in the gut, and alleviated epithelial damage and inflammatory infiltrates in enteric mucosa and submucosa of the GI tract.

Conclusions

In this *Brief Review*, we have discussed recent advances in engineered oral formulations for modulating the gut microbiota and host immune responses. These engineering strategies improved pharmacokinetics of drugs and allow for the precise delivery of therapeutic cargo to the GI tract. However, despite the great strides being made regarding modulating the immune system with gut microbiota-derived biomaterials in preclinical models, clinical translation of these therapeutics still poses challenges. When developing the next generation oral formulations, their biosafety profiles and scalability need to be addressed. Moreover, the complex and bidirectional interactions between biomaterials, the gut microbiome, and the immune system in the context of specific diseases remain unclear. New technologies, such as artificial intelligence and machine learning, may contribute to identifying microbial signatures that are implicated in various diseases. Additionally, the spatial diversity of the gut microbiome along the GI tract calls for more investigations, as current research often evaluates the gut microbiota as a whole. Building a better understanding of spatial interaction among biomaterials, the gut microbiome, and immune cells will provide guidelines for newly emerging engineering strategies. Finally, various gut microbiota-associated factors, such as microbial enzymes, microbial-derived Ags, and lipids, are emerging as new adjuvants, Ags, or agonists to affect host immunity, and they may provide new opportunities for the next generation of oral formulations.

Disclosures

J.J.M. declares financial interests for board membership, as a paid consultant, for research funding, and/or as an equity holder in EVOQ Therapeutics and Saros Therapeutics. The University of Michigan has a financial interest in EVOQ Therapeutics, Inc. The other authors have no financial conflicts of interest.

References

1. Kallus, S. J., and L. J. Brandt. 2012. The intestinal microbiota and obesity. *J. Clin. Gastroenterol.* 46: 16–24.
2. Maynard, C. L., C. O. Elson, R. D. Hatton, and C. T. Weaver. 2012. Reciprocal interactions of the intestinal microbiota and immune system. *Nature* 489: 231–241.
3. Feehley, T., C. H. Plunkett, R. Bao, S. M. Choi Hong, E. Culleen, P. Belda-Ferre, E. Campbell, R. Aitoro, R. Nocerino, L. Paparo, et al. 2019. Healthy infants harbor intestinal bacteria that protect against food allergy. *Nat. Med.* 25: 448–453.
4. Finnie, I. A., A. D. Dwarakanath, B. A. Taylor, and J. M. Rhodes. 1995. Colonic mucin synthesis is increased by sodium butyrate. *Gut* 36: 93–99.
5. Shan, M., M. Gentile, J. R. Yeiser, A. C. Walland, V. U. Bornstein, K. Chen, B. He, L. Cassis, A. Bigas, M. Cols, et al. 2013. Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals. *Science* 342: 447–453.
6. Schröder, J.-M. 1999. Epithelial peptide antibiotics. *Biochem. Pharmacol.* 57: 121–134.
7. Zheng, L., C. J. Kelly, K. D. Battista, R. Schaefer, J. M. Lanis, E. E. Alexeev, R. X. Wang, J. C. Onyiah, D. J. Kominsky, and S. P. Colgan. 2017. Microbial-derived butyrate promotes epithelial barrier function through IL-10 receptor–dependent repression of claudin-2. *J. Immunol.* 199: 2976–2984.
8. Geuking, M. B., Y. Köller, S. Rupp, and K. D. McCoy. 2014. The interplay between the gut microbiota and the immune system. *Gut Microbes* 5: 411–418.
9. Burr, A. H. P., A. Bhattacharjee, and T. W. Hand. 2020. Nutritional modulation of the microbiome and immune response. *J. Immunol.* 205: 1479–1487.
10. Singer, J. R., E. G. Blosser, C. L. Zindl, D. J. Silberger, S. Conlan, V. A. Laufer, D. DiToro, C. Deming, R. Kumar, C. D. Morrow, et al. 2019. Preventing dysbiosis of the neonatal mouse intestinal microbiome protects against late-onset sepsis. *Nat. Med.* 25: 1772–1782.
11. Shi, Y., W. Zheng, K. Yang, K. G. Harris, K. Ni, L. Xue, W. Lin, E. B. Chang, R. R. Weichselbaum, and Y.-X. Fu. 2020. Intratumoral accumulation of gut microbiota facilitates CD47-based immunotherapy via STING signaling. *J. Exp. Med.* 217: e20192282.
12. Ferrari, V., and M. Rescigno. 2023. The intratumoral microbiota: friend or foe? *Trends Cancer* 9: 472–479.
13. Gopalakrishnan, V., C. N. Spencer, L. Nezi, A. Reuben, M. C. Andrews, T. V. Karpinets, P. A. Prieto, D. Vicente, K. Hoffman, S. C. Wei, et al. 2018. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 359: 97–103.
14. Matson, V., J. Fessler, R. Bao, T. Chongsuwat, Y. Zha, M.-L. Alegre, J. J. Luke, and T. F. Gajewski. 2018. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science* 359: 104–108.
15. Routy, B., E. Le Chatelier, L. Derosa, C. P. M. Duong, M. T. Alou, R. Daillère, A. Fluckiger, M. Messaoudene, C. Rauber, M. P. Roberti, et al. 2018. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 359: 91–97.
16. Kim, S. M., J. R. DeFazio, S. K. Hyoju, K. Sangani, R. Keskey, M. A. Krezalek, N. N. Khodarev, N. Sangwan, S. Christley, K. G. Harris, et al. 2020. Fecal microbiota transplant rescues mice from human pathogen mediated sepsis by restoring systemic immunity. *Nat. Commun.* 11: 2354.
17. Schmidt, T. S. B., J. Raes, and P. Bork. 2018. The human gut microbiome: from association to modulation. *Cell* 172: 1198–1215.
18. Schultz, M. 2008. Clinical use of *E. coli* Nissle 1917 in inflammatory bowel disease. *Inflamm. Bowel Dis.* 14: 1012–1018.
19. Kurtz, C. B., Y. A. Millet, M. K. Puurunen, M. Perreault, M. R. Charbonneau, V. M. Isabella, J. W. Kotula, E. Antipov, Y. Dagon, W. S. Denney, et al. 2019. An engineered *E. coli* Nissle improves hyperammonemia and survival in mice and shows dose-dependent exposure in healthy humans. *Sci. Transl. Med.* 11: eaau7975.
20. Luke, J. J., S. A. Piha-Paul, T. Medina, C. F. Verschraegen, M. Varterasian, A. M. Brennan, R. J. Riese, A. Sokolovska, J. Strauss, D. L. Hava, and F. Janku. 2023. Phase I study of SYNB1891, an engineered *E. coli* nissle strain expressing STING agonist, with and without atezolizumab in advanced malignancies. *Clin. Cancer Res.* 29: 2435–2444.
21. Puurunen, M. K., J. Vockley, S. L. Searle, S. J. Sacharow, J. A. Phillips III, W. S. Denney, B. D. Goodlett, D. A. Wagner, L. Blankstein, M. J. Castillo, et al. 2021. Safety and pharmacodynamics of an engineered *E. coli* Nissle for the treatment of phenylketonuria: a first-in-human phase 1/2a study. [Published erratum appears in 2021 *Nat. Metab.* 4: 1214.] *Nat. Metab.* 3: 1125–1132.
22. Cubillos-Ruiz, A., T. Guo, A. Sokolovska, P. F. Miller, J. J. Collins, T. K. Lu, and J. M. Lora. 2021. Engineering living therapeutics with synthetic biology. *Nat. Rev. Drug Discov.* 20: 941–960.
23. Koh, E., I. Y. Hwang, H. L. Lee, R. De Sotto, J. W. J. Lee, Y. S. Lee, J. C. March, and M. W. Chang. 2022. Engineering probiotics to inhibit *Clostridioides difficile* infection by dynamic regulation of intestinal metabolism. *Nat. Commun.* 13: 3834.
24. Zou, Z.-P., Y. Du, T.-T. Fang, Y. Zhou, and B.-C. Ye. 2023. Biomarker-responsive engineered probiotic diagnoses, records, and ameliorates inflammatory bowel disease in mice. *Cell Host Microbe* 31: 199–212.e5.
25. Scott, B. M., C. Gutiérrez-Vázquez, L. M. Sanmarco, J. A. da Silva Pereira, Z. Li, A. Plasencia, P. Hewson, L. M. Cox, M. O'Brien, S. K. Chen, et al. 2021. Self-tunable engineered yeast probiotics for the treatment of inflammatory bowel disease. *Nat. Med.* 27: 1212–1222.
26. Yue, Y., J. Xu, Y. Li, K. Cheng, Q. Feng, X. Ma, N. Ma, T. Zhang, X. Wang, X. Zhao, and G. Nie. 2022. Antigen-bearing outer membrane vesicles as tumour vaccines produced in situ by ingested genetically engineered bacteria. *Nat. Biomed. Eng.* 6: 898–909.
27. Chen, Y. E., D. Bousbaine, A. Veinbachs, K. Atabakhsh, A. Dimas, V. K. Yu, A. Zhao, N. J. Enright, K. Nagashima, Y. Belkaid, and M. A. Fischbach. 2023. Engineered skin bacteria induce antitumor T cell responses against melanoma. *Science* 380: 203–210.
28. Praveschotinunt, P., A. M. Duraj-Thatte, I. Gelfat, F. Bahl, D. B. Chou, and N. S. Joshi. 2019. Engineered *E. coli* Nissle 1917 for the delivery of matrix-tethered therapeutic domains to the gut. *Nat. Commun.* 10: 5580.
29. Li, S., W. Jiang, C. Zheng, D. Shao, Y. Liu, S. Huang, J. Han, J. Ding, Y. Tao, and M. Li. 2020. Oral delivery of bacteria: basic principles and biomedical applications. *J. Control. Release* 327: 801–833.
30. Xu, C., Q. Ban, W. Wang, J. Hou, and Z. Jiang. 2022. Novel nano-encapsulated probiotic agents: encapsulate materials, delivery, and encapsulation systems. *J. Control. Release* 349: 184–205.
31. Yang, X., J. Yang, Z. Ye, G. Zhang, W. Nie, H. Cheng, M. Peng, K. Zhang, J. Liu, Z. Zhang, and J. Shi. 2022. Physiologically inspired mucin coated *Escherichia coli* Nissle 1917 enhances biotherapy by regulating the pathological microenvironment to improve intestinal colonization. *ACS Nano* 16: 4041–4058.
32. Zhou, J., M. Li, Q. Chen, X. Li, L. Chen, Z. Dong, W. Zhu, Y. Yang, Z. Liu, and Q. Chen. 2022. Programmable probiotics modulate inflammation and gut microbiota for inflammatory bowel disease treatment after effective oral delivery. *Nat. Commun.* 13: 3432.
33. Guo, H., W.-C. Chou, Y. Lai, K. Liang, J. W. Tam, W. J. Brickey, L. Chen, N. D. Montgomery, X. Li, L. M. Bohannon, et al. 2020. Multi-omics analyses of radiation survivors identify radioprotective microbes and metabolites. *Science* 370: eaay9097.
34. Pan, J., G. Gong, Q. Wang, J. Shang, Y. He, C. Catania, D. Birnbaum, Y. Li, Z. Jia, Y. Zhang, et al. 2022. A single-cell nanocoating of probiotics for enhanced amelioration of antibiotic-associated diarrhea. *Nat. Commun.* 13: 2117.
35. Liu, J., S. Lane, R. Lall, M. Russo, L. Farrell, M. Debreli Coskun, C. Curtin, R. Araujo-Gutierrez, M. Scherrer-Crosbie, B. H. Trachtenberg, et al. 2022. Circulating hemopexin modulates anthracycline cardiac toxicity in patients and in mice. *Sci. Adv.* 8: eadc9245.
36. Fábrega, M.-J., A. Rodríguez-Nogales, J. Garrido-Mesa, F. Algieri, J. Badía, R. Giménez, J. Gálvez, and L. Baldomà. 2017. Intestinal anti-inflammatory effects of outer membrane vesicles from *Escherichia coli* Nissle 1917 in DSS-experimental colitis in mice. *Front. Microbiol.* 8: 1274.
37. Xu, J., J. Xu, T. Shi, Y. Zhang, F. Chen, C. Yang, X. Guo, G. Liu, D. Shao, K. W. Leong, and G. Nie. 2023. Probiotic-inspired nanomedicine restores intestinal

homeostasis in colitis by regulating redox balance, immune responses, and the gut microbiome. *Adv. Mater.* 35: e2207890.
38. Zhang, W., C. R. Mackay, and M. E. Gershwin. 2023. Immunomodulatory effects of microbiota-derived short-chain fatty acids in autoimmune liver diseases. *J. Immunol.* 210: 1629–1639.
39. Kovatcheva-Datchary, P., A. Nilsson, R. Akrami, Y. S. Lee, F. De Vadder, T. Arora, A. Hallen, E. Martens, I. Björck, and F. Bäckhed. 2015. Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of *Prevotella*. *Cell Metab.* 22: 971–982.
40. Schoeler, M., and R. Caesar. 2019. Dietary lipids, gut microbiota and lipid metabolism. *Rev. Endocr. Metab. Disord.* 20: 461–472.
41. Spencer, C. N., J. L. McQuade, V. Gopalakrishnan, J. A. McCulloch, M. Vetizou, A. P. Cogdill, M. A. W. Khan, X. Zhang, M. G. White, C. B. Peterson, et al. 2021. Dietary fiber and probiotics influence the gut microbiome and melanoma immunotherapy response. *Science* 374: 1632–1640.
42. Li, Q., K. E. Goggin, J. Seo, and N. Egilmez. 2022. Dietary low-fiber promotes resistance to immune checkpoint inhibitor immunotherapy in the LSL-KrasG12D lung cancer model. *J. Immunol.* 208(Suppl.): 120.101.
43. Lam, K. C., R. E. Araya, A. Huang, Q. Chen, M. Di Modica, R. R. Rodrigues, A. Lopès, S. B. Johnson, B. Schwarz, and E. Bohrnsen. 2021. Microbiota triggers STING-type I IFN-dependent monocyte reprogramming of the tumor microenvironment. *Cell* 184: 5338–5356.e21.
44. Carmody, R. N., J. E. Bisanz, B. P. Bowen, C. F. Maurice, S. Lyalina, K. B. Louie, D. Treen, K. S. Chadaideh, V. Maini Rekdal, E. N. Bess, et al. 2019. Cooking shapes the structure and function of the gut microbiome. *Nat. Microbiol.* 4: 2052–2063.
45. Li, L.-L., Y.-T. Wang, L.-M. Zhu, Z.-Y. Liu, C.-Q. Ye, and S. Qin. 2020. Inulin with different degrees of polymerization protects against diet-induced endotoxemia and inflammation in association with gut microbiota regulation in mice. *Sci. Rep.* 10: 978.
46. Beukema, M., M. M. Faas, and P. de Vos. 2020. The effects of different dietary fiber pectin structures on the gastrointestinal immune barrier: impact via gut microbiota and direct effects on immune cells. *Exp. Mol. Med.* 52: 1364–1376.
47. Shepherd, E. S., W. C. DeLoache, K. M. Pruss, W. R. Whitaker, and J. L. Sonnenburg. 2018. An exclusive metabolic niche enables strain engraftment in the gut microbiota. *Nature* 557: 434–438.
48. Cao, Y., and R. Mezzenga. 2020. Design principles of food gels. *Nat. Food* 1: 106–118.
49. Han, K., J. Nam, J. Xu, X. Sun, X. Huang, O. Animasahun, A. Achreja, J. H. Jeon, B. Pursley, N. Kamada, et al. 2021. Generation of systemic antitumour immunity via the in situ modulation of the gut microbiome by an orally administered inulin gel. *Nat. Biomed. Eng.* 5: 1377–1388.
50. Aggarwal, N., A. M. E. Breedon, C. M. Davis, I. Y. Hwang, and M. W. Chang. 2020. Engineering probiotics for therapeutic applications: recent examples and translational outlook. *Curr. Opin. Biotechnol.* 65: 171–179.
51. Fong, W., Q. Li, and J. Yu. 2020. Gut microbiota modulation: a novel strategy for prevention and treatment of colorectal cancer. *Oncogene* 39: 4925–4943.
52. Lee, J. W., C. T. Y. Chan, S. Slomovic, and J. J. Collins. 2018. Next-generation biocontainment systems for engineered organisms. *Nat. Chem. Biol.* 14: 530–537.
53. Koirala, S., and A. K. Anal. 2021. Probiotics-based foods and beverages as future foods and their overall safety and regulatory claims. *Future Foods* 3: 100013.
54. Van den Abbeele, P., S. Deyaert, C. Thabuis, C. Perreau, D. Bajic, E. Wintergerst, M. Joossens, J. Firrman, D. Walsh, and A. Baudot. 2023. Bridging preclinical and clinical gut microbiota research using the ex vivo SIFR® technology. *Front. Microbiol.* 14: 1131662.
55. Swanson, K. S., G. R. Gibson, R. Hutkins, R. A. Reimer, G. Reid, K. Verbeke, K. P. Scott, H. D. Holscher, M. B. Azad, N. M. Delzenne, and M. E. Sanders. 2020. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nat. Rev. Gastroenterol. Hepatol.* 17: 687–701.
56. Zhang, T., J. Zhang, and L. Duan. 2023. The role of genetically engineered probiotics for treatment of inflammatory bowel disease: a systematic review. *Nutrients* 15: 1566.
57. Yang, W., and Y. Cong. 2021. Gut microbiota-derived metabolites in the regulation of host immune responses and immune-related inflammatory diseases. *Cell. Mol. Immunol.* 18: 866–877.
58. Noto, D., and S. Miyake. 2022. Gut dysbiosis and multiple sclerosis. *Clin. Immunol.* 235: 108380.
59. Wang, Q., Y. Luo, K. Ray Chaudhuri, R. Reynolds, E.-K. Tan, and S. Pettersson. 2021. The role of gut dysbiosis in Parkinson's disease: mechanistic insights and therapeutic options. *Brain* 144: 2571–2593.
60. Pérez-Reytor, D., C. Puebla, E. Karahanian, and K. García. 2021. Use of short-chain fatty acids for the recovery of the intestinal epithelial barrier affected by bacterial toxins. *Front. Physiol.* 12: 650313.
61. Zou, F., Y. Qiu, Y. Huang, H. Zou, X. Cheng, Q. Niu, A. Luo, and J. Sun. 2021. Effects of short-chain fatty acids in inhibiting HDAC and activating p38 MAPK are critical for promoting B10 cell generation and function. *Cell Death Dis.* 12: 582.
62. Bachem, A., C. Makhlouf, K. J. Binger, D. P. de Souza, D. Tull, K. Hochheiser, P. G. Whitney, D. Fernandez-Ruiz, S. Dähling, and W. Kastenmüller. 2019. Microbiota-derived short-chain fatty acids promote the memory potential of antigen-activated $CD8^+$ T cells. *Immunity* 51: 285–297.e5.
63. Schiweck, C., S. Edwin Thanarajah, M. Aichholzer, S. Matura, A. Reif, E. Vrieze, A. Weigert, and A. Visekruna. 2022. Regulation of $CD4^+$ and $CD8^+$ T cell biology by short-chain fatty acids and its relevance for autoimmune pathology. *Int. J. Mol. Sci.* 23: 8272.
64. Kostic, A. D., R. J. Xavier, and D. Gevers. 2014. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology* 146: 1489–1499.
65. Levi, I., M. Gurevich, G. Perlman, D. Magalashvili, S. Menascu, N. Bar, A. Godneva, L. Zahavi, D. Chermon, and N. Kosower. 2021. Potential role of indolelactate and butyrate in multiple sclerosis revealed by integrated microbiome-metabolome analysis. *Cell Rep. Med.* 2: 100246.
66. Shubitowski, T. B., B. G. Poll, N. Natarajan, and J. L. Pluznick. 2019. Short-chain fatty acid delivery: assessing exogenous administration of the microbiome metabolite acetate in mice. *Physiol. Rep.* 7: e14005.
67. Gill, P. A., M. C. van Zelm, J. G. Muir, and P. R. Gibson. 2018. Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. *Aliment. Pharmacol. Ther.* 48: 15–34.
68. Azienda Sanitaria ASL Avellino 2. 2017. Effects of short-chain fatty acids on inflammatory and metabolic parameters in maintenance hemodialysis. In: ClinicalTrials.gov. National Library of Medicine (US), Bethesda, MD. NLM Identifier: NCT02976688. Available at: https://classic.clinicaltrials.gov/show/NCT02976688. Accessed: June 12, 2023.
69. Clinical Nutrition Research Center, Illinois Institute of Technology. 2022. ButyraGen, short chain fatty acids and gut microbiome. In: ClinicalTrials.gov. National Library of Medicine (US), Bethesda, MD. NLM Identifier: NCT05601635. Available at: https://classic.clinicaltrials.gov/show/NCT05601635. Accessed: June 12, 2023.
70. Imperial College London. 2013. Oral propionate to treat and prevent diabetes. In: ClinicalTrials.gov. National Library of Medicine (US), Bethesda, MD. NLM Identifier: NCT01692002. Available at: https://classic.clinicaltrials.gov/show/NCT01692002. Accessed: June 12, 2023.
71. Shashni, B., Y. Tajika, and Y. Nagasaki. 2021. Design of enzyme-responsive short-chain fatty acid-based self-assembling drug for alleviation of type 2 diabetes mellitus. *Biomaterials* 275: 120877.
72. Wang, R., S. Cao, M. E. H. Bashir, L. A. Hesser, Y. Su, S. M. C. Hong, A. Thompson, E. Culleen, M. Sabados, N. P. Dylla, et al. 2023. Treatment of peanut allergy and colitis in mice via the intestinal release of butyrate from polymeric micelles. *Nat. Biomed. Eng.* 7: 38–55.
73. Sharma, J., T. Banerjee, M. Naithani, N. Kumar, S. K. Singh, and S. Basu. 2023. Gut microbiome and Crohn's disease: an enigmatic crosstalk. *IntechOpen.* Available at: http://dx.doi.org/10.5772/intechopen.110768.
74. Wei, S., M. I. Bahl, S. M. D. Baunwall, J. F. Dahlerup, C. L. Hvas, and T. R. Licht. 2022. Gut microbiota differs between treatment outcomes early after fecal microbiota transplantation against recurrent *Clostridioides difficile* infection. *Gut Microbes* 14: 2084306.
75. Yan, L., L. Wang, S. Gao, C. Liu, Z. Zhang, A. Ma, and L. Zheng. 2020. Celery cellulose hydrogel as carriers for controlled release of short-chain fatty acid by ultrasound. *Food Chem.* 309: 125717.
76. Scottish Universities Environmental Research Centre. 2015. RCT to describe the effects of colon delivered acetate, propionate and butyrate on satiety and glucose homeostasis. In: ClinicalTrials.gov. National Library of Medicine (US), Bethesda, MD. NLM Identifier: NCT02451189. Available at: https://classic.clinicaltrials.gov/show/NCT02451189. Accessed: June 18, 2023.
77. University of Glasgow. 2015. Impact of exercise combined with inulin propionate ester on fat oxidation. In: ClinicalTrials.gov. National Library of Medicine (US), Bethesda, MD. NLM Identifier: NCT04016350. Available at: https://classic.clinicaltrials.gov/show/NCT04016350. Accessed: June 18, 2023.
78. Imperial College London. 2008. Effect of fibre products on appetite and weight. In: ClinicalTrials.gov. National Library of Medicine (US), Bethesda, MD. NLM Identifier: NCT00750438. Available at: https://classic.clinicaltrials.gov/show/NCT00750438. Accessed: June 18, 2023.
79. Imperial College London. 2017. Colonic propionate, appetite, and weight loss. In: ClinicalTrials.gov. National Library of Medicine (US), Bethesda, MD. NLM Identifier: NCT03322514. Available at: https://classic.clinicaltrials.gov/show/NCT03322514. Accessed: June 18, 2023.
80. Polyviou, T., K. MacDougall, E. S. Chambers, A. Viardot, A. Psichas, S. Jawaid, H. C. Harris, C. A. Edwards, L. Simpson, K. G. Murphy, et al. 2016. Randomised clinical study: inulin short-chain fatty acid esters for targeted delivery of short-chain fatty acids to the human colon. *Aliment. Pharmacol. Ther.* 44: 662–672.
81. Chambers, E. S., C. S. Byrne, D. J. Morrison, K. G. Murphy, T. Preston, C. Tedford, I. Garcia-Perez, S. Fountana, J. I. Serrano-Contreras, E. Holmes, et al. 2019. Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: a randomised cross-over trial. *Gut* 68: 1430–1438.
82. Hill, D. R., S. P. Kessler, H. K. Rho, M. K. Cowman, and C. A. de la Motte. 2012. Specific-sized hyaluronan fragments promote expression of human β-defensin 2 in intestinal epithelium. *J. Biol. Chem.* 287: 30610–30624.
83. Rayahin, J. E., J. S. Buhrman, Y. Zhang, T. J. Koh, and R. A. Gemeinhart. 2015. High and low molecular weight hyaluronic acid differentially influence macrophage activation. *ACS Biomater. Sci. Eng.* 1: 481–493.
84. Lee, Y., K. Sugihara, M. G. Gillilland III, S. Jon, N. Kamada, and J. J. Moon. 2020. Hyaluronic acid-bilirubin nanomedicine for targeted modulation of dysregulated intestinal barrier, microbiome and immune responses in colitis. *Nat. Mater.* 19: 118–126.
85. Zhang, Z., X. Wu, S. Cao, M. Cromie, Y. Shen, Y. Feng, H. Yang, and L. Li. 2017. Chlorogenic acid ameliorates experimental colitis by promoting growth of *Akkermansia* in mice. *Nutrients* 9: 677.
86. Zheng, F., Z. Wang, C. Stanton, R. P. Ross, J. Zhao, H. Zhang, B. Yang, and W. Chen. 2021. *Lactobacillus rhamnosus* FJSYC4-1 and *Lactobacillus reuteri* FGSZY33L6 alleviate metabolic syndrome via gut microbiota regulation. *Food Funct.* 12: 3919–3930.

87. Cheng, S., H. Shen, S. Zhao, Y. Zhang, H. Xu, L. Wang, B. Di, L. Xu, and C. Hu. 2020. Orally administered mesoporous silica capped with the cucurbit[8]uril complex to combat colitis and improve intestinal homeostasis by targeting the gut microbiota. *Nanoscale* 12: 15348–15363.
88. Ouyang, J., B. Deng, B. Zou, Y. Li, Q. Bu, Y. Tian, M. Chen, W. Chen, N. Kong, T. Chen, and W. Tao. 2023. Oral hydrogel microbeads-mediated in situ synthesis of selenoproteins for regulating intestinal immunity and microbiota. *J. Am. Chem. Soc.* 145: 12193–12205.
89. Mourtada, R., H. D. Herce, D. J. Yin, J. A. Moroco, T. E. Wales, J. R. Engen, and L. D. Walensky. 2019. Design of stapled antimicrobial peptides that are stable, nontoxic and kill antibiotic-resistant bacteria in mice. *Nat. Biotechnol.* 37: 1186–1197.
90. Chen, P. B., A. S. Black, A. L. Sobel, Y. Zhao, P. Mukherjee, B. Molparia, N. E. Moore, G. R. Aleman Muench, J. Wu, W. Chen, et al. 2020. Directed remodeling of the mouse gut microbiome inhibits the development of atherosclerosis. *Nat. Biotechnol.* 38: 1288–1297.
91. Liu, S., R. M. Rezende, T. G. Moreira, S. K. Tankou, L. M. Cox, M. Wu, A. Song, F. H. Dhang, Z. Wei, and G. Costamagna. 2019. Oral administration of miR-30d from feces of MS patients suppresses MS-like symptoms in mice by expanding *Akkermansia muciniphila*. *Cell Host Microbe* 26: 779–794.e8.
92. Liu, S., A. P. da Cunha, R. M. Rezende, R. Cialic, Z. Wei, L. Bry, L. E. Comstock, R. Gandhi, and H. L. Weiner. 2016. The host shapes the gut microbiota via fecal microRNA. *Cell Host Microbe* 19: 32–43.
93. Behrouzi, A., F. Ashrafian, H. Mazaheri, A. Lari, M. Nouri, F. Riazi Rad, Z. Hoseini Tavassol, and S. D. Siadat. 2020. The importance of interaction between microRNAs and gut microbiota in several pathways. *Microb. Pathog.* 144: 104200.
94. Teng, Y., Y. Ren, M. Sayed, X. Hu, C. Lei, A. Kumar, E. Hutchins, J. Mu, Z. Deng, and C. Luo. 2018. Plant-derived exosomal microRNAs shape the gut microbiota. *Cell Host Microbe* 24: 637–652.e8.
95. Bungau, S., D. M. Tit, T. Behl, L. Aleya, and D. C. Zaha. 2021. Aspects of excessive antibiotic consumption and environmental influences correlated with the occurrence of resistance to antimicrobial agents. *Curr. Opin. Environ. Sci. Health* 19: 100224.
96. Guk, J., J. Guedj, C. Burdet, A. Andremont, J. de Gunzburg, A. Ducher, and F. Mentré. 2021. Modeling the effect of DAV132, a novel colon-targeted adsorbent, on fecal concentrations of moxifloxacin and gut microbiota diversity in healthy volunteers. *Clin. Pharmacol. Ther.* 109: 1045–1054.
97. Lee, J. S., P. Han, R. Chaudhury, S. Khan, S. Bickerton, M. D. McHugh, H. B. Park, A. L. Siefert, G. Rea, J. M. Carballido, et al. 2021. Metabolic and immunomodulatory control of type 1 diabetes via orally delivered bile-acid-polymer nanocarriers of insulin or rapamycin. [Published erratum appears in 2021 *Nat. Biomed. Eng.* 5: 1403.] *Nat. Biomed. Eng.* 5: 983–997.
98. Zhang, G., Q. Wang, W. Tao, W. Jiang, E. Elinav, Y. Wang, and S. Zhu. 2022. Glucosylated nanoparticles for the oral delivery of antibiotics to the proximal small intestine protect mice from gut dysbiosis. *Nat. Biomed. Eng.* 6: 867–881.
99. Mu, H., H. Bai, F. Sun, Y. Liu, C. Lu, Y. Qiu, P. Chen, Y. Yang, L. Kong, and J. Duan. 2019. Pathogen-targeting glycovesicles as a therapy for salmonellosis. *Nat. Commun.* 10: 4039.
100. Liu, J., Y. Wang, W. J. Heelan, Y. Chen, Z. Li, and Q. Hu. 2022. Mucoadhesive probiotic backpacks with ROS nanoscavengers enhance the bacteriotherapy for inflammatory bowel diseases. *Sci. Adv.* 8: eabp8798.