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Genetic Factors and the Intestinal Microbiome Guide Development of Microbe-based Therapies for Inflammatory Bowel Diseases

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Abstract

The intestinal microbiota is a dynamic community of bacteria, fungi, and viruses that mediates mucosal homeostasis and physiology. Imbalances in the microbiome and aberrant immune responses to gut bacteria can disrupt homeostasis and are associated with inflammatory bowel diseases (IBD) in humans and colitis in mice. We review genetic variants associated with IBD and their effects on the intestinal microbiome, the immune response, and disease pathogenesis. The intestinal microbiome, which includes microbial antigens, adjuvants, and metabolic products, affects the development and function of the intestinal mucosa and inflammatory responses in the gut. Strategies to manipulate the microbiome might therefore be used in treatment of IBD. We review microbe-based therapies for IBD and the potential to engineer patients' intestinal microbiota. We discuss how studies of patients with IBD and mouse models have advanced our understanding of the interactions between genetic factors and the gut microbiome, and challenges to development of microbe-based therapies for IBD.

Inflammatory bowel diseases (IBD) include Crohn's disease (CD) and ulcerative colitis (UC)— chronic diseases that develop via complex interactions among genetic, immune, environmental, and microbial factors.[1–3] Dysregulation of any components of this network

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can result in intestinal inflammation and IBD. Genetic studies identified regulators of this network that are altered in patients with IBD—many of these control the immune response to microbes.[4, 5] Variants associated with risk for IBD have been identified in *NOD2*, *ATG16L1*, *CARD9*, and *CLEC7A*.[6] Variants in genes that control immune detection of and response to microbes can perturb intestinal homeostasis and promote intestinal inflammation. It is important to distinguish factors that mediate the immune response to pathogens from factors that control the overall microbial ecology, which can also be affected by environmental factors (diet, medications, geography).[7] However, as we study the mechanisms by which genetic variants associated with IBD affect responses to microbes, we might learn more about environmental factors that also do so, and identify new targets for diagnosis and treatment of IBD.

Studies from model systems have indicated that the gut microbiome can be modified to increase or reduce the severity of intestinal inflammation. The gut microbiome can be altered by introduction of microbes or their effectors, such as lipids, small molecules, proteins, or sugars. Over the last decade, interest in microbe-based therapies has increased due to the number and perceived safety of these therapies, as well as the potential to correct one of the causes of a disease, rather than the symptoms. Increased interest in these therapies is partly due to insights from studies of antibiotics, probiotics, and more recently fecal microbial transplantation (FMT), for IBD and other disorders.[8] Strategies to correct the microbiome or its functions in patients with UC or CD have produced inconsistent results, although antibiotics were found to be effective in patients with pouchitis, with an excellent safety profile.[9–11] FMT was found to reduce symptoms in some patients with UC, although outcomes varied. Further studies are required to optimize selection of donors, determine the ability of the donated microbiota to engraft, and determine whether FMT might be better as an induction or maintenance therapy.

Studies are also needed to determine how variants in genes whose products function in microbe sensing pathways (such as NOD2) would affect microbial therapies. We review interactions between IBD-associated gene variants and the microbiome, and strategies to therapeutically target specific microbiome functions.[9, 12] The growth of microbe-based therapies presents new challenges to drug development and regulatory approval.

Genes That Regulate the Microbiome

Genes encode many proteins that microbes are exposed to, as well as the availability of nutrients and the level of the immune response to microbes.[13–16] Genome-wide association studies of patients with IBD have identified variants in genes that affect the intestinal response to microbes (Figure 1).[6, 17–25]

Variants in NOD2

Variants in *NOD2* were the first to be associated with risk of CD. *NOD2* encodes an intracellular pattern recognition receptor, which interacts with peptidoglycan motifs of bacteria.[26, 2728] NOD2 helps control pathogenic bacteria through hematopoietic and non-hematopoietic cells. It was initially believed that individuals with some variants of *NOD2* were unable to efficiently clear bacterial pathogens, leading to IBD pathology.[29] This

hypothesis is supported by the association in patient cohorts between gastrointestinal pathogens and IBD onset.[30] However, it became apparent that NOD2 also mediates the immune response to non-pathogenic, commensal microbes. Patients with variants in *NOD2* have microbiomes that are distinct from individuals without these variants, characterized by increased abundance of *Escherichia* species and reduced *Faecalibacterium* species, though this pattern can also be independent of *NOD2* variants.[15, 31–33] *Nod2*$^{-/-}$ mice have intestinal dysbiosis, which increase their susceptibility to colitis, compared with wild-type mice.[32, 34–39] Researchers have identified commensal bacteria that are pathogenic in *Nod2*$^{-/-}$ mice (pathobionts), such as *Bacteroides vulgatus*. Mucosal barrier defects observed in *Nod2*$^{-/-}$ mice were linked to *B vulgatus*, including abnormalities in goblet cells, expression of inflammatory genes, and increased numbers of intraepithelial lymphocytes that express IFN gamma.[37] Depletion of *B vulgatus* reversed the mucosal barrier defects in *Nod2*$^{-/-}$ mice, so targeted removal of organisms that exacerbate NOD2 signaling defects might restore intestinal barrier functions in patients with IBD.

Variants in ATG16L1 and autophagy

Several variants associated with risk of CD are in genes that regulate the autophagy pathway (such as *ATG16L1, IGRM*, and *LRRK2*). Autophagy has many functions, but one of its effects is to mediate lysosomal degradation and clearance of intracellular bacteria.[18, 19, 40] Several studies have demonstrated that NOD2 interacts with ATG16L1 and that expression of CD-associated variants disrupts association between these proteins, impairing bacterial clearance and antigen presentation.[41, 42] The variant encoding the T300A substitution in *ATG16L1* increases susceptibility of the gene product to caspase-3 cleavage and reduces its function.[43] Similar to *Nod2*$^{-/-}$ mice, mice hypomorphic for *ATG16L1* have microbiota-dependent susceptibility to induction of colitis, as well as defects in toll-like receptor (TLR) signaling and production of antimicrobial peptides by Paneth cells. These abnormalities in TLR signaling and Paneth cell function have also been observed in patients with CD who are homozygous for the T300A substitution in *ATG16L1*.[44, 45] In mice, disruption of the *ATG16L1* gene affects CD4^{+} T cells, reducing numbers of intestinal Foxp3^{+} T-regulatory (Treg) cells and T-helper 2 (Th2) cell-mediated responses. These impaired T-cell functions contribute to disruption of the mucosal barrier, via loss of tolerance to intestinal antigens and increased production of IgG and IgA against commensal microbiota.[46] Although many individuals carry IBD-associated variants in *NOD2* and genes that regulate autophagy, only a small proportion develop IBD. Additional environmental factors and alterations to interactions between the intestinal epithelia and microbiota are therefore likely to be required for development of IBD.

Studies of mice and cells with deletion of ATG16L1 or NOD2 have found these proteins to mediate the effects of therapeutic microbes, by blocking immunomodulatory signals. For example, the common human commensal *Bacteroides fragilis* reduces colitis in mice by converting CD4+ T cells into Foxp3+ T-regulatory (Treg) cells that produce IL10.[47, 48] This effect of *B fragilis* is lost when dendritic cells are defective in either NOD2 or ATG16L1 signaling.[49] Human immune cells that express ATG16L1 T300A do not induce Treg cell development upon exposure to *B fragilis*.

Variants in CLEC7A and CARD9

Bacteria are the most well-defined microbes in the intestinal microbiota, but fungal communities are also altered in microbiomes of patients with IBD. This should not be surprising considering the associations between IBD and an aberrant immune response to fungal antigens, based on detection of antibodies to *Saccharomyces cerevisiae*.[50] Similar to the intestinal bacterial communities in patients with IBD, the diversity of fungal microbiota is decreased and certain atypical phyla dominate, such Ascomycota and Basidiomycota.[51] Fungal members of the gut microbiota interact with pattern recognition receptors such as CLEC7A (also called DECTIN1), a glycoprotein expressed by cells in the innate immune system that recognizes a beta-1,3-linked and beta-1,6-linked glucans from fungi. A single-nucleotide polymorphism in *CLEC7A* has been associated with IBD. Mice lacking DECTIN1 have increased susceptibility to colitis and an altered fungal community.[52] Interestingly, some variants in *CLEC7A* have been associated with medically refractory ulcerative colitis; no other IBD-associated variants have been associated with response to therapy.[52]

DECTIN1 signals through the adaptor protein caspase recruitment domain containing protein 9 (CARD9).[23] *Card9*$^{-/-}$ mice also have an altered fungal community structure with increased susceptibility to dextran sodium sulfate (DSS)-induced colitis.[53] The fungal dysbiosis that results from loss of CARD9 in mice is associated with loss of Th17 cells, consistent with the importance of these cells to controlling fungal infections. Patients with a homozygous mutation in *CARD9* (rs10781499) have severe mucocutaneous candidiasis.[53–5556] DECTIN1 signaling via CARD9 might alter the immune response through changes in pathways regulated by NFκB, JNK, and MAP kinase.[54]

CARD9 also has a role in response to bacteria through its interaction with NOD2.[57] A study of *Card9*$^{-/-}$ mice reported alterations in fungal and bacterial communities, but colitis susceptibility was dependent only on the bacterial community. Variations in phenotypes of knockout mice reveals the complexities of microbiome functions; many potential confounding variables affect host microbial interactions. In *Card9*$^{-/-}$ mice, bacterial tryptophan metabolites were found to account for some of variations in phenotypes.[58] Bacterial tryptophan metabolites signal through human aryl hydrocarbon receptors (AHR), which are important for mucosal tolerance. Impaired microbial tryptophan metabolism in *Card9*$^{-/-}$ mice was associated with colitis susceptibility. Administration of *Lactobacillus* strains that metabolize tryptophan into AHR ligands was sufficient to reduce colitis in *Card9*$^{-/-}$ mice. Fecal samples from patients with CD or UC with IBD-associated polymorphisms in *CARD9* lacked AHR ligands.

Human Leukocyte Antigens (HLAs)

HLA are encoded by genes in the major histocompatibility complex (MHC), among the most polymorphic in humans. Proteins encoded by the MHC locus mediate antigen presentation and coordination of the immune response. The diversity of HLAs allows the immune system to respond to a variety of pathogens, but certain polymorphisms increase risk for inappropriate responses to self-antigens.[59] Variants in MHC class II genes have been associated with UC and CD and correlate with disease location.[60] The heterozygosity of

variants in class II genes is lower in patients with UC.[61] These variants might reduce recognition of antigens on commensal microbes.[61] Studies of mice and patients with other MHC class II-associated diseases, including rheumatoid arthritis, celiac disease, and ankylosing spondylitis,[62–6566–68] revealed a correlation between specific HLA alleles and distinct communities of microbes, which increase numbers of Th17 cells and intestinal permeability.[69, 70] Studies of mice indicate that interactions between HLA polymorphisms and the intestinal microbiota are mediated by altered production of IgA and specific bacterial species, such as *Bacteroides* spp, which are sufficient to induce colitis.[64, 71] Further research is needed into HLA variants and their associated microbial communities in patients with IBD.

Mucins

The intestinal mucus layer serves as another physical barrier that separates luminal microbes from the intestinal epithelium. The mucus layer comprises the glycoproteins MUC2, MUC5AC, MUC5B, and MUC6, which are secreted by goblet cells.[72] Specific microbes degrade mucin glycoproteins, so microbial community structure corresponds with changes in mucin glycosylation. The diverse array of mucin glycans create a specific niche for specific intestinal bacteria that have evolved to bind these glycoproteins and use them as a carbon source. A disruption in the mucus barrier would therefore change bacterial ecology and deplete an important intestinal barrier function. In intestines of mice with colitis caused by administration of DSS or disruption of the *Il10* gene, and in patients with UC, the mucus layer is thinner and highly penetrable to organisms that do not typically inhabit this niche.[73, 74] Patients with UC have aberrant expression of MUC5AC, MUC6, and MUC2.[75, 7677, 78] Mice with deletion of MUC2 have increased susceptibility to colitis.[79–81] Although variants in mucin genes have not been associated with IBD, their altered expression patterns in intestinal tissues from patients with IBD indicate that their activity is important for maintenance of the microbiome and prevention of inflammation.

Other genetic variants

Genome-wide association studies have identified over 200 loci with significant associations with IBD, but only a minority of these loci can be mapped to the 1 or 2 most likely alleles.[82] These loci are generally enriched for protein-altering variants and proteins in cytokine pathways. For example, the IL23 signaling pathway includes IL23R, IL12B, TYK2, and JAK2. IL23 promotes development of Th17 cells, CD4+ T cells, Tc17 CD8+ T cells, and innate lymphoid cells, type 3. Agents that block IL12 and IL23 signaling have been approved by the Food and Drug Administration (FDA) for treatment of CD. However, cytokines produced by Th17 cells, including IL17 and IL22, protect against IBD.[83, 84] In mice, IL17 prevents expansion of segmented filamentous bacteria (SFB), which promote colitis, via IL17 receptor (IL17R)-mediated epithelial cell signaling or by increasing neutrophil recruitment.[85, 86] Some of the anti-inflammatory effects of IL22 are mediated by increased control of intestinal pathobionts and/or increased production of mucus.[87, 88] Although many treatments for IBD aim to control cytokine production, dietary and bacterial metabolites can be ligands for G-protein coupled receptors (GPCR), which often activate anti-inflammatory signaling pathways.[89, 90] Variants in GPCR genes have been associated

with IBD. The GPCR GPR35 a receptor for the tryptophan intermediate kynurenic acid, which has been associated to IBD and primary sclerosing cholangitis.[82, 89, 91]

Interactions Between Genetic Variants and the Intestinal Microbiome

Altered interactions between the intestinal epithelium and the microbiota are an important step in IBD pathogenesis.[92] These defective interactions might be corrected with microbes or microbial products. Many variants in genes associated with IBD affect responses of immune or intestinal cells to microbes, but do not affect the overall microbial ecology. However, we are currently able to assess only large changes in the overall microbiome, and we might miss changes in specific microbial populations or niches. We need to better understand interactions between genetic alterations and changes in specific populations of microbes. It is also important to conduct experiments with appropriate controls, because many factors can perturb the microbiome.[93] The most powerful evidence for the mechanisms by which genetic variants alter the intestinal microbiome has come from studies of adoptive fecal transfer with littermate controls and careful analyses of knockout or knockdown mice, such as in the studies of NOD2.

Studies of mice have shown that alterations of the microbiota can promote colitis. Microbes interact with cell surface proteins, secreted metabolites, and other environmental substrates (Figure 2). It is not clear however, whether it is alterations in the microbes themselves or their effectors that promote development of IBD. Understanding how different intestinal microbes can cause different phenotypes of IBD could lead to development of microbe-based therapies.

Germ-free and gnotobiotic mice

Studies of germ-free and gnotobiotic mice have increased our understanding of interactions between intestinal cells, microbes, and development of the immune system. Mice raised under germ-free conditions have alterations in gut-associated lymphoid tissue, plasma cells, T cells, responses to microbial peptides, the crypt–villus architecture, and the mucus barrier.[94, 95] Germ-free mice also have reduced expression of NOD2, indicating that its expression is regulated in response to microbes.[34] Colonization of germ-free mice with a healthy microbiota restored intestinal homeostasis, although individual mucosal functions can be restored by organisms such as *Lactobacillus plantarum* or *E coli* Nissle 1917, which reactivates NOD2 signaling.[34]

Interestingly, infection with norovirus can restore most mucosal barrier abnormalities observed in germ-free mice.[96] Experiments with germ-free and gnotobiotic mice established the role of the microbiota in development of colitis independent of genetic factors. In mice with T-cell transfer induced colitis, specific microbes promote development of colitis whereas others do not.[97] Microbes can induce or reduce the severity of colitis in IL10-knockout mice or in mice given DSS.[98–100] So, individual populations of microbes can either promote or prevent intestinal inflammation, depending on genotype; in IL10 knockout mice, most bacteria elicit colitis. Microbe-based therapies might therefore be selected based on a patient's genotype, but not be effective in the entire population of patients with IBD.

However, some microbiome therapies, such as those that increase mucosal barrier function, could have the widest applicability.

Interactions between microbes and intestinal cells

Intestinal microbes can alter the immune response. For example, commensal Clostridia strains promote accumulation of Foxp3$^+$ Treg cells in the gut by inducing production of transforming growth factor beta and indoleamine 2,3-dioxygenase.[101, 102] Treg cells downregulate inflammatory responses and mice colonized with specific species of Clostridia are resistant to induction of colitis.[101] Although specific species of Clostridia can induce development of ROR0γt$^+$ Treg cells, *Clostridium ramosum* also has this function.[103, 104] Studies of gnotobiotic mice identified specific immune-modulatory effects of individual species of commensal bacteria and showed that specific types of immune cells, such as Treg cells can be induced by a wide range of bacteria whereas others appear to require specific microbes.[105] Some microbes activate populations of immune cells that promote intestinal inflammation, such as Th17 cells.[106] SFB activate Th17 cells in intestines of mice; adherent invasive *E coli* (AIEC) and *Bifidobacteria adolescentis* induce mucosal and systemic populations of Th17 cells in the gut.[107–109] Reduction of Th17 cell-inducing bacteria can reduce the severity of colitis in mice.

An organism does not have to change its abundance in the population to have significant effects. SFB, which increases development of Th17 cells, also promotes T-cell dependent production of IgA.[110] Coating of microbes by IgA has been proposed as a marker of immune activation by that microbe; IgA-coated bacteria, including certain *Enterobacteriaceae*, induce colitis in mice and have been associated with CD-associated spondyloarthritis.[108, 110, 111] As IBD-promoting pathobionts may be specific to a gene or an individual, understanding IgA responses to microbes might help prevent the emergence of pathobionts or help us target pathobionts in specific individuals. The immune response to a microbe is likely specific to its niche. *Alcaligenes, Achromobacter, Bordetella*, and *Ochrobactrum* spp. specifically colonize lymphoid tissues, where they interact with innate lymphoid cells and dendritic cells to modulate IL10 production and intestinal repair mechanisms.[112] The bioactivity of a microbe might require certain environmental signals, such as dietary metabolites, which can induce production or activity of bacterial effectors, or serve as metabolic substrates. Some *Alisepes*, *Clostridium*, and *Bilophila* spp can decrease production of tumor necrosis factor (TNF) by immune cells only in the presence of a certain diet.[113114]

Specific species of Candida can cause colitis in mice; colonization can be inhibited by *Bacteroides thetaiotamicron*, which induce the production of antimicrobial peptide CRAMP.[115] Interactions among organisms might affect the efficacy of microbiota-based therapies. Helminths are not considered commensals, but were prevalent during human history and are believed to have functions that affect microbiome development.[116] Certain helminths induce responses of Th2 cells and increase IL10 secretion.[117–119] Although helminths probably regulate these immune responses to promote their own infection of a patient, their functions might benefit patients with IBD, which has been demonstrated in phase 2 and 3 studies. The observation that the same organism can be beneficial or detrimental, depending on the

patient or model, is not unique to helminths, but applies to many microbes. Viruses could have roles in IBD pathogenesis and norovirus is sufficient to restore mucosal barrier defects in germ-free mice, independent of microbiota.[96, 120]

Microbe effectors

Studies of model systems have identified organisms that induce specific cell responses, but these responses vary. Many complex factors mediate these interactions and we know little about the mechanisms by which bacteria alter the intestinal environment. Microbes interact with the intestinal epithelium or each other via secreted or cell-surface effectors. Identifying these effectors could help us learn more about the pathogenesis of IBD and lead to therapeutic strategies. Small molecules produced by microbes have tested for their therapeutic effects for decades.[121] Studies are needed to identify the effectors produced by microbes that act on intestinal and immune cells.[122]

Short-chain fatty acids (SCFAs) reduce colitis, promote Treg-cell development, and downregulate of inflammatory signaling pathways.[123, 124] Clostridia species produce SCFAs, which reach millimolar concentrations in the intestine and can activate GPCRs, inhibit histone deacetylases, and provide an energy source for colon epithelial cells.[125–127] Polyamines such as putrescine or spermidine are virulence factors but also enhance intestinal barrier functions including mucus secretion, T-cell differentiation, and production of IgA.[128–131] *Bifidobacterium animalis* increases polyamine levels, which correlates with decreased secretion of TNF and IL6 by myeloid cells.[130] Interestingly, bacteria and human cells each produce polyamines, which might mediate some of their interactions.

Bacteria and human cells also each produce long-chain *N*-acyl signaling molecules that signal via specific GPCRs to regulate immunity, inflammation, and metabolism.[90, 132] Structural similarity between human and bacterial signaling molecules is likely to be common, because bacteria are also able to synthesize the neurotransmitter GABA and certain *Bacteroides* metabolize tryptophan to tryptamine, a precursor to serotonin.[133–135] Bacterial trypthophan metabolites are ligands for AHR, but the metabolism of tryptophan and other aromatic amino acids in Clostridia has been linked to intestinal barrier functions through the production of indoleproprionic acid.[136] Bacterial metabolites of bile acids, such as the generation of taurine, might regulate inflammasome functions and increase microbial diversity.[137]

Bacteria interact through cell-surface effectors, including via secretion of outer membrane vesicles.[138] Zwitterionic polysaccharide A (PSA), on the surface of *B fragilis*, regulates activity of Foxp3$^+$ Treg cells in the gut.[47] Administration of purified PSA, or *B fragilis*, is sufficient to activate intestinal Treg cells and reduce colitis in mice.[48, 49, 139] Lipopolysaccharide (LPS), probably the most well-studied bacterial cell surface molecule, has countless variations in structure. Specific types of LPS, such as penta-acylated LPS produced by certain *Bacteroides*, can inhibit immune responses, in contrast to hexa-acylated LPS from *E coli*, which stimulates the immune response.[140] Interestingly, in a mouse model of diabetes, this LPS from *E coli* reduced autoimmunity and development of diabetes. Therefore, bacteria can have different effects in different model systems, so it is important to understand all the effects of a microbe before it is included in a therapeutic strategy.

Sphingolipids isolated from *Bacteroides fragilis* are similar to the human molecules and can regulate natural killer T cells.[141]

Challenges to Microbiome-based Therapeutics

Microbe-based therapeutic strategies can aim to alter the overall microbiome or its environment, introduce therapeutic microbes, or alter production of microbe effectors. Early studies focused on application of therapeutic microbes, despite the challenges of developing a drug that includes living organisms. Small-molecule development begins with basic research and discovery of bioactive molecules, followed by preclinical studies (formulation, toxicity, and pharmacokinetic analyses), followed by trials of safety and efficacy in patients. Development of microbe-based therapies has changed concepts of drug mechanisms, formulation, and monitoring, requiring new approaches for development and regulation (Figure 3). In 2012 the Center for Biologics Evaluation and Research issued guidelines to assist in therapeutic development of live organisms, which they classified as live biotherapeutic products (LBPs). LBPs are defined as biologic products that contain live organisms, such as bacteria, and that might be used in prevention, treatment, or cure of human diseases but are not vaccines. Development of prebiotics and bacterial effectors is likely to follow regular drug development pathways, but LBPs are the most pursued of the microbe-based therapies (Figure 3).

FDA Regulation

In 2012 the FDA published regulations for development of LBPs, which were updated in 2016.[142] It is important to distinguish LBPs from probiotics, which are organisms that have obtained the generally recognized as safe label and fall outside of this regulation. Trials of LBPs for treatment of diseases requires an investigational new drug (IND) application. An IND application can be waived for an LBP that is available in conventional foods or dietary supplements, in consultation with the FDA. FMTs were first performed without an IND because feces were considered to be widely available, but in 2012, regulation by the FDA changed— now an IND is required for studies of FMT in patients with IBD. However, use of FMT for recurrent *C difficile* infection (CDI) has discretionary regulation. The FDA does not require toxicity studies for trials of FMT, but it does require adequate characterization of microbe strains to be tested. Chemistry, manufacturing, and control data must include the historical context of the organism(s), the purity, and details about the presence of virulence factors, toxins or antibiotic resistance genes and the potential to spread these genes.

Whole-genome sequencing is performed for many LBPs to address these safety concerns. Genetically modified LBP require additional tests, to ensure the stability of genetic modifications. Antibiotic resistance genes may be present but require justification and are not acceptable for LBPs that could cause opportunistic infections. Phenotype-based antibiotic resistance testing for each LBP is required in addition to traditional toxicological profiling. Product release testing (the identity, viability, potency and purity of each LBP) is perhaps more important step, relative to small molecule therapies, because LBPs are a challenge to quantify and can change during production. Potency assays (such as colony-forming units) should be used to calculate the dose or release of a predefined product when

mechanisms are well defined. Later stages of might require an assay that tests the agent's mechanism, which might not be straightforward for a complex LBP. In addition, it is still not clear what level of evidence is needed to justify a specific selection of strains for an LBP. Regulations are likely to include standardization of these definitions among countries, because there is no equivalent definition of an LBP in the European Union, which has categorizations for therapeutic organisms not present in the United States.

Microbiota-based Therapeutics in Preclinical and Clinical Development

As the guiding framework for LBP has been clarified over last 6 years there has been an increase in companies developing discovery platforms and/or introducing candidate therapies into pre-clinical and clinical studies for the treatment of IBD (Table 1).

Modulators of the microbiome

The microbiome can be modified by improving mucosal barrier functions or depletion or enrichment of organisms linked with diseases, an increased or decreased immune response, or other outcomes. Zoenasa is a formulation of *N*-acetyl cysteine, phosphatidylcholine, and mesalamine that is believed to strengthen the mucosal barrier. Zoenasa has been formulated as a rectal gel or oral tablet and tested in a phase 2 study of patients with distal UC. Specific microbes or communities of microbes may be pathogens (pathobionts), and depletion of these microbes might be beneficial. A challenge to modulating the microbiome by depleting pathobionts is that these microbes may be specific to an individual based on their pathophysiology and/or genetics. Production of IgA in response to bacteria might be measured to identify specific pathobionts in individual patients.

Immuron is an oral immunotherapy (antigens, adjuvants, antibodies) designed to reduce or increase specific microbiota. IMM124E is an oral formulation of antibody against LPS and glycosphingolipid adjuvants that is preclinical studies but might be used to treat UC. BiomX directly targets pathobionts using bacteriophages and Eligo depletes bacteria by using CRISPR.[143] The companies that are developing these agents have not revealed their specific target species, but Eligo has a platform to allow for an individualized assessment of potential pathobionts. Ecoactive has just entered phase 1 and 2 trials of patients with CD—it is an oral bacteriophage cocktail that depletes AIEC. AIEC is enriched by defects in NOD2 signaling in patients with CD.[144] EB-8018 was designed to reduce AIEC by blocking fimH. EB-8018 is entering phase 2 studies of patients with CD and was found to be safe in a phase 1 study. A diagnostic assay (IBD-210) has been designed to measure fimH in fecal samples, to identify patients likely to respond to EB-8018.

LBPs

Studies of patients and animal models have led to the discovery of many LBPs. LBP are being developed using a variety of formulations, including naturally derived communities, defined communities, individual organisms, and genetically modified organisms. SER287 is a naturally derived community and SER301 a defined community based on human cohort studies whereas VE202 was developed as a synthetic community in mice, based on a targeted increases in Treg cells.[101] In contrast to community LBP, Thetanix is a single strain

of *Bacteroides thetaiotamicron* that is in a phase 1 trial of children with CD. *B thetaiotamicron* might have multiple mechanisms, including modulation of fungi, although there is also a negative association between *B thetaiotamicron* and infection with pathogenic strains of *E coli*.

The pleiotropic effects of LBP will warrant specific safety attention. A strain of *Clostridium butyricum* is in preclinical studies for IBD was found to be safe for treatment of CDI. *C butyricum* is believed to act specifically by increasing SCFAs, though other LBPs are also believed to increase SCFA.[145] ImmuneBiotech has a narrow focus on a proprietary panel of lactobacilli, to which they assign immunomodulatory functions. Many lactobacilli carry generally recognized as safe designations, which will facilitate their approval process and are easy to manipulate in the laboratory for the development of genetically modified organisms. AG-014 is a lactobacillus engineered to produce a nanobody against TNF that is in phase 1 studies of patients with IBD.

Countries outside the United States, have an additional regulatory category, independent of dietary supplement and LBP, which is a food for special medical purposes. A food for special medical purposes is naturally found in the diet but can be marketed for the treatment of a disease. In Denmark, profermin is a food for special medical purposes—it is a combination of *Lactobacillus plantarum*, oats, and phosphatidylcholine. Each component of this pill has a separate effect as a prebiotic (oats), barrier modulator (phosphatidylcholine), and LBP (*L plantarum*) though it is unclear if there is interaction among components. Profermin is on the market and has been studied in small trials of patients with UC, in which it had moderate efficacy but without endoscopic endpoints.[146, 147] Helminths, specifically *Trichuris suis*, showed efficacy in phase 2 studies of patients with CD but had limited efficacy in phase 3 studies.[148–151]

Microbe effectors

Microbe effectors have specific effects on cells and follow a traditional drug development strategy. Approval of microbial effectors by regulatory agencies might be straightforward, but there are few products in early stages of development, because we understand so little about them. EB110 is a microbe-derived metabolite identified in humans that has been associated with development of CD, via unknown mechanisms. SG-2–0776 is a microbe effector (protein) that promotes intestinal healing and is in preclinical studies for treatment of IBD. PSA has been one of the most extensively studied bacterial effectors and has a number of immune-regulatory properties.[47, 152] However, IBD-associated variants in *NOD2* and *ATG16L1* could mitigate the effect of PSA and be used to identify patients not likely to respond.[49] PSA is in preclinical development and it is unclear whether clinical trials will compare effects in patients with different genotypes.

Formulation strategies

Formulation is an important challenge for microbiome-based therapies. Formulation aims for reproducible effects among individuals and delivery of viable organisms to their niche. Many LBPs in early-stage trials are administered daily, because it is likely that LBPs do not incorporate into the microbiome and expand. However, they might be given in intermittent

or even single doses, if our understanding of microbiome homeostasis improves, and we can more carefully select LBPs or use of adjuvants.

Companies have focused on formulations for better delivery an LBP to a niche. The Gemicel capsule was developed for colonic release of LBP, incorporating 2 separate pH dependent mechanisms. Aquashell is a pH-sensitive formulation for colonic delivery that incorporates a separate polysaccharide coating that is digested by colon microbes. Both formulation strategies have been used to encapsulate LBPs that are in trials of patients with IBD. Duocoat is optimized for duodenal release, using a pH-sensitive coat, and Phloral is optimized for colon release. Each of these encapsulation strategies necessitate specific attention to a strict anaerobic process that is unique for each LBP, because previous dietary probiotics (such as *Lactobacillus* or *Bifidobacterium*) were microaerophilic.[153] Genetically modified organisms for treatment of IBD might be viewed as a type of formulation strategy to increase a therapeutic effect and mitigate variation. Genetic tools to manipulate human microbes are required. Synlogic programs internal circuits in *E coli* Nissle strains, to induce expression of effector genes in response to specific environmental signals. This technology is used to produce microbial effectors only in the correct environment. Synlogic has also developed chromosome markers for in vivo monitoring of LBPs, which will facilitate bioavailability studies.

Targeting interactions between intestinal cells and microbes

We have begun to identify cell signaling pathways that regulated by bacteria and might be therapeutically manipulated. SGM-1019 is a small molecule that affects the inflammasome, identified using a discovery platform. SGM-1019 has progressed through phase I studies and is being developed for treatment of IBD and non-alcoholic steatohepatitis, for which it is entering phase 2 studies.

Future Directions

Microbe-based therapies are becoming more diverse and effective as our understanding of the interactions between the microbiome and human cells increases. We have begun to better understand the effects of genetic and environmental factors on the microbiome and its products or effectors. Formulation strategies can be refined to address the primary challenge of diversity in the microbiomes among individuals and for treatment of specific diseases.

Additional challenges to development and use of microbe-based therapies involves issues regarding intellectual property. A full discussion of this topic is beyond the scope of this review, but one of the biggest problems is how to enforce patent laws for the composition of a natural product. LBPs that can be defined as natural products include isolated microbes or their effectors (such as metabolites or proteins). Natural products can be protected by method patents, which state their use for treatment of IBD, but these patents are not as easily enforced, which dissuades companies from developing these types of products. One strategy has been to genetically modify microbes or use them in combination with other microbes, as a genetically modified organism or a defined community that is not found in nature (not a natural product). Companies have obtained composition of matter patents for LBPs that are natural communities, so it will be important to see how these patents are enforced if these

products come to market. Patent law protection is critical for development of microbe-based therapies; it is likely that the legal framework will change as it has in the past.

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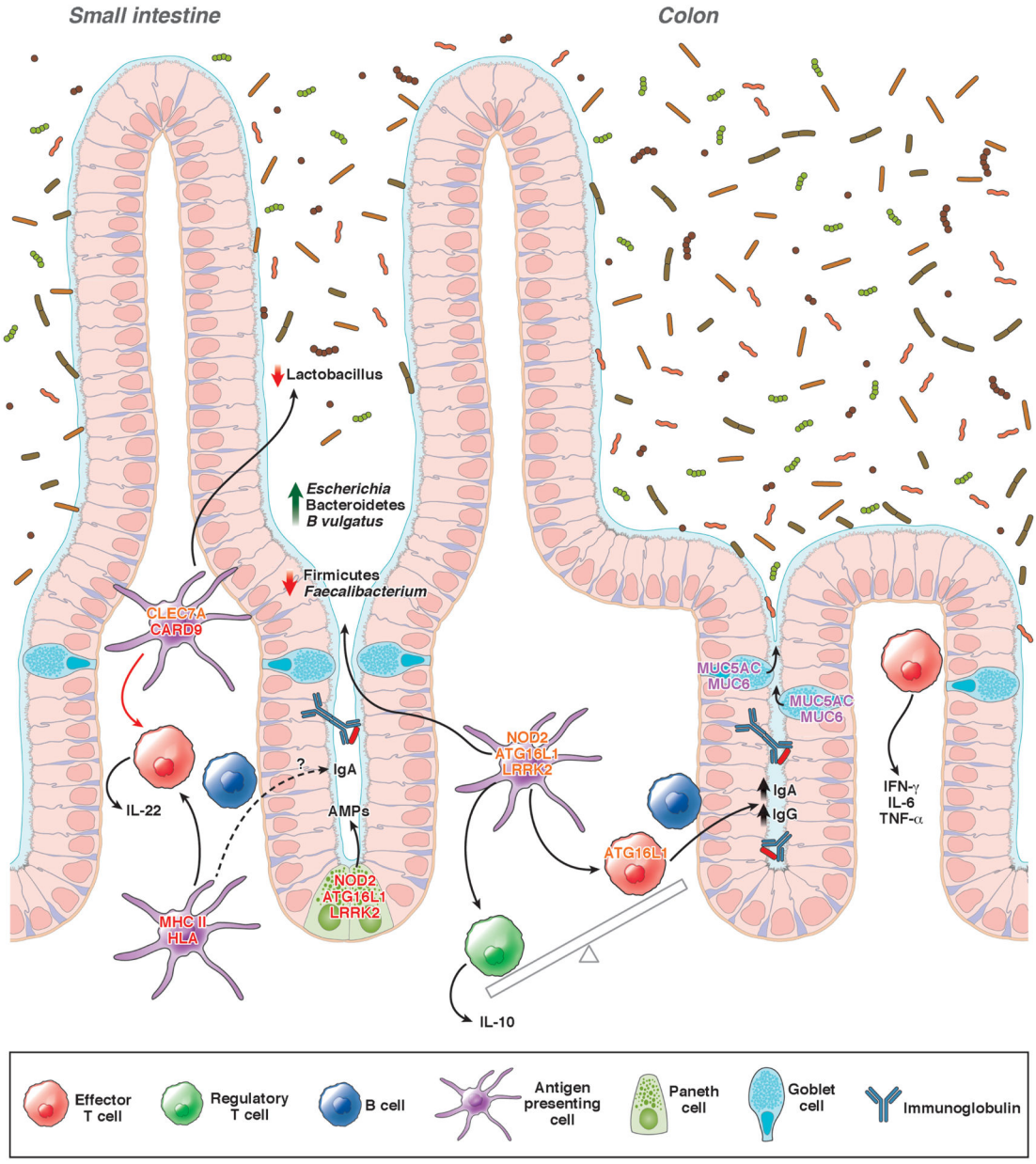

Figure 1. Genetic factors that affect the intestinal microbiome.

Variants genes that affect risk for IBD have been associated with alterations in the composition of the microbiome. Mutations in *NOD2*, *ATG16L1*, and *LRRK2* reduce secretion of antimicrobial peptide (AMP) by Paneth cells. Variants in *CLEC7A* and *CARD9* have been associated with decreased abundance of *Lactobacillus*, possibly due to altered activities of dendritic cells and macrophages. Variants in *NOD2* are associated with increased abundance of *Escherichia* species and *Bacteroides vulgatus* and reductions in *Faecalibacterium* species. Impaired ATG16L1 signaling has been associated with increased production of IgG and IgA against commensal microbiota, resulting in a loss of tolerance to intestinal microbes. Polymorphisms in MHC class II or HLA genes affect production of IgA in response to microbes. Defects in mucus production alter the intestinal microbiome and

increase susceptibility to colitis. Gene names in red have variants associated with CD and UC; gene names in orange have variants associated with only CD; and gene names in purple have variants associated with only UC.

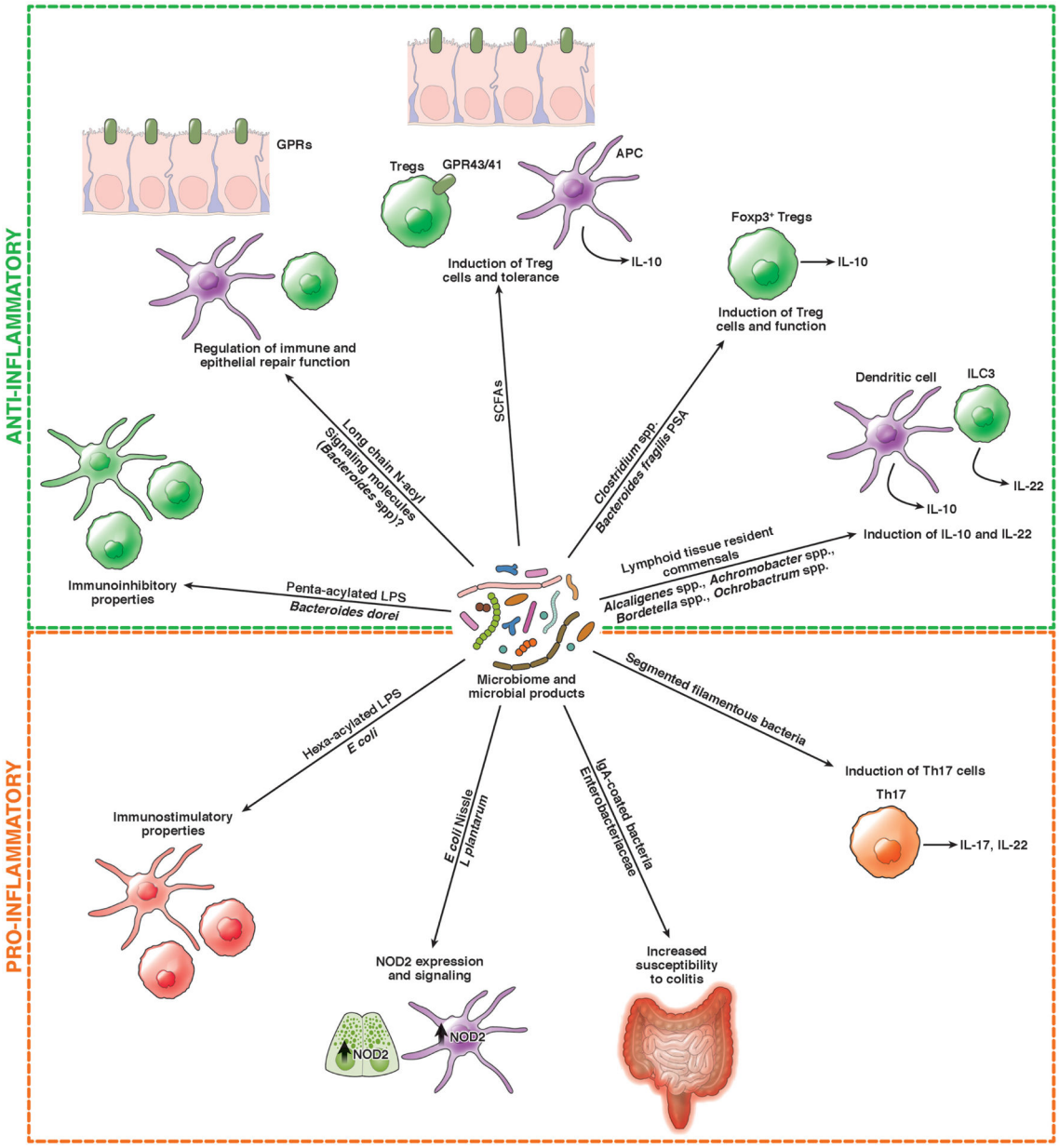

Figure 2. Effects of the Microbiome on Intestinal and Immune Cells.

The intestinal microbiome and its products modulate immune responses, via induction of dendritic cells (DCs) and lymphocytes (such as Th17 cells, Treg cells [Tregs in figure], and innate lymphoid cells (ILCs)), and cytokine production (IL10, IL22). Intestinal bacteria can also modulate immune signaling pathways, such as expression of NOD2, and epithelial repair. Specific microbes can increase susceptibility of mice to colitis.

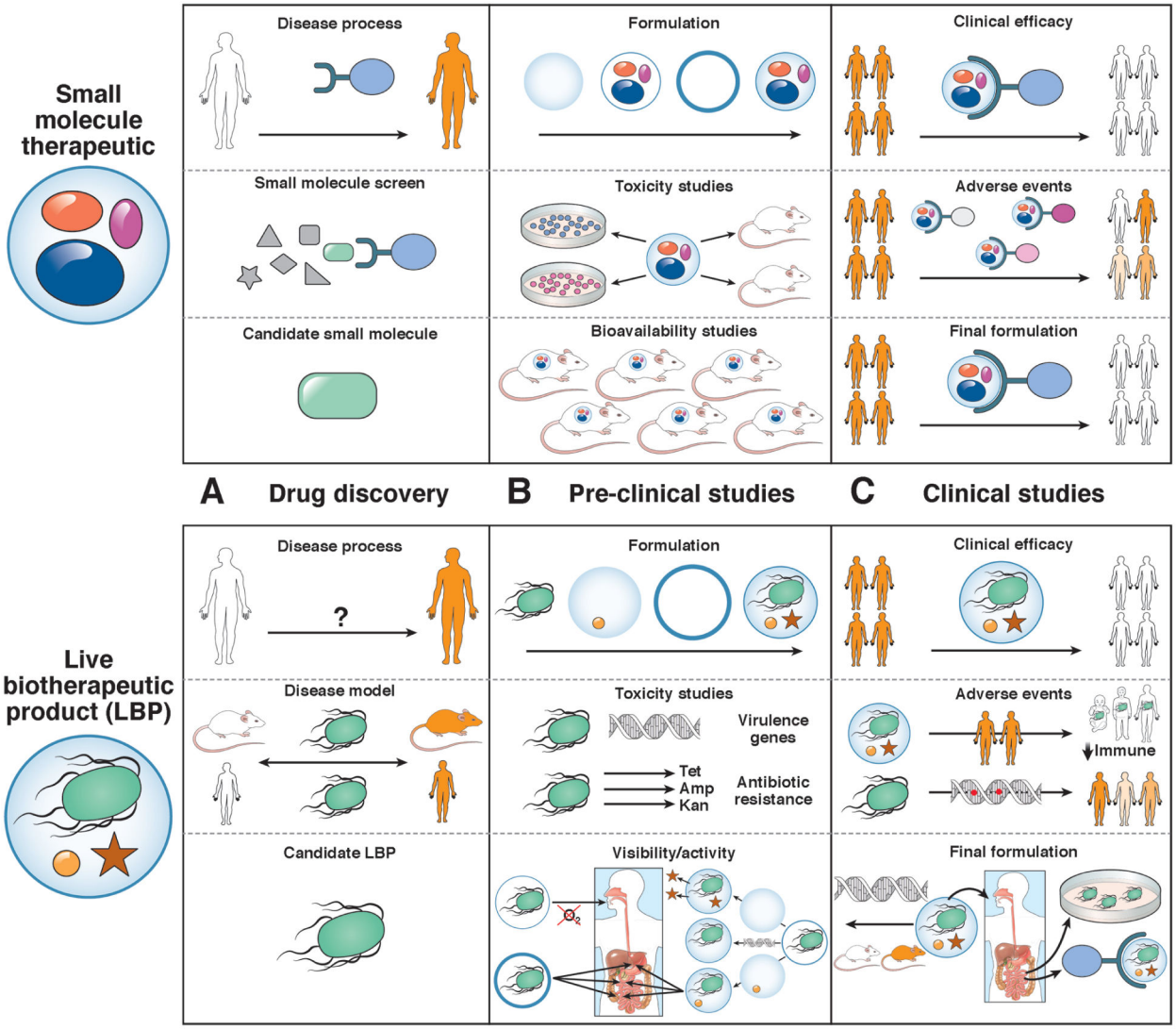

Figure 3. Challenges to Development of Therapeutic LBPs.

Compared with small molecules, LBPs have different challenges at each stage of drug development. Challenges are presented to drug discovery, preclinical studies, and clinical studies. A. In contrast to small molecules, which usually target a specific protein or class of feature of proteins, LBPs are identified based on their association with a disease phenotype in humans or mouse models. B. Small molecules require extensive toxicity studies, whereas LBPs are believed to be non-toxic but require assessments for virulence or antibiotic resistance. Preclinical studies of LBPs are not informative for bioavailability, but focus on viability or bioactivity, which can require specific encapsulation methods, adjuvants, or genetic modifications. C. Trials of LBPs require specific attention to adverse events related to transmission of the microbe, loss of its bioactivity, or off-target effects. Small molecules can also have off target effects, but these may be easier to predict, based on finding from preclinical studies. Early-phase studies of LPBs might be important for final formulation,

because bioavailability and potential mechanisms can be assessed based on findings from small groups of patients.

Table 1.

Microbiota-based Therapies in Development for IBD

Therapy Type	Company	Location	Product*	Delivery*	Development Stage	Mechanism
Ecology modulator	Enterome	USA	EB-8018 small molecule	oral	phase 2 study of patients with CD	prevents pathobiont invasion by blocking FIMH
	Immuron	Australia	N/A	oral	preclinical	antibodies and adjuvant to boost immune response to pathobionts
	BiomX	Israel	BX002	N/A	preclinical	bacteriophage to deplete pathobionts
	Eligo	France	N/A	N/A	preclinical	Crispr-CAS to deplete pathobionts
	Intralytix	USA	EcoActive	oral	phase 1 and 2a study of patients with CD	bacteriophage to target AIEC
	Artizan Biosciences	USA	N/A	N/A	Preclinical	Subtractive therapy
Live Biotherapeutic Product	Seres Therapeutics	USA	SER287 naturally derived community	oral	phase 2 study of patients with UC	not available
	Seres Therapeutics Janssen (Vendanta License)	USA	SER301 defined community	oral	preclinical	not available
			VE202 defined community	oral	phase 1 study of patients with UC	Induce Treg cells
	Rebiotix (acquired by Ferring)	USA	RBX2660	enema	phase 1 in pediatric patients with UC	Restore microbiome composition
	4D Pharma	UK	Thetanix B *thetaiotamicron*	oral	phase 1 study of patients with CD	not available
	4D Pharma Osel	UK	Rosburix *R hominis*	oral	preclinical	not available
		USA	CBM588 *C butyricum*	oral	phase 1	Increase SCFAs
	ImmuneBiotech	Sweden	IB002 Lactobacilli	N/A	preclinical	not available
	Actobiotic	USA	AG-014 GMO	oral	phase 1	heterologous expression of anti-TNF nanobody by Lactobacillus
	Rise Therapeutics	USA	R-3750 GMO	N/A	preclinical	Lactobacillus for heterologous expression
	Nordisk Rebalance	Denmark	Profermin *L latarum* oats phosphatidylcholine	oral	marketed as food for special medical purpose	phase 2 of patients with UC, a prebiotic that increases SCFAs
	Finch Therapeutics	USA	FIN524, defined community	oral	preclinical studies of colitis	not available
	Allergan	Ireland	ABI-M201,301	oral	preclinical	licensed from Assembly Biosciences and uses Gemicel coating
	NextBiotix/Exeliom Biosciences	FRA	NBX-1650	n/a	Preclinical	F. prausnitzii to treat inflammation

Therapy Type	Company	Location	Product*	Delivery*	Development Stage	Mechanism
	ViThera	USA	VT301	Oral	Preclinical	Modified Lactobacilli strains
	Chain Biotech	USA	CHN-1, CHN-2	Oral	preclinical	anti-microbial peptide with Clostridium Assisted Drug Delivery
	PanTheryx	USA	PTX-400	Oral	Preclinical	Medical food - prebiotic
Microbial effectors	Host Therabiomics	UK/USA	L1173	N/A	preclinical	platform to identify effectors
	Second Genome	USA	SG-2–0776 protein	oral	preclinical	intestinal healing
	Symbiotix Biotherapies	USA	SYMB-104 polysaccharide A	N/A	preclinical	*B fragilis*-derived immune modulator
	Alma Bio Therapeutics	FRA	N/A Plasmid	Injection	Preclinical	Plasmids that produce Heat Shock Proteins
	Enterome	USA	EB110/EB220	oral	preclinical	microbial metabolite associated with CD
Formulation	Finch Therapeutics	USA	aquashell	oral	N/A	pH release polysaccharide
	Intract Pharma	UK	phloral duocoat	oral	N/A	pH release
	Prodigest	Belgium	in vitro microbiome model	N/A	N/A	predict in vivo conditions
	Synlogic	USA	genetically modified organism	N/A	N/A	Not available
	Assembly	USA	gemicel capsule	oral	N/A	pH release
Host Target	Second Genome	USA	SGM-1019 Small molecule	oral	phase 1	modulates inflammasome

N/A, not applicable because agent is in preclinical stage of development

Note: Companies were identified by the Janssen Human Microbiome Institute from public resources.