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# Manipulation of Microbiome, a Promising Therapy for Inflammatory Bowel Diseases

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## Abstract

Inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, are featured by chronic intestinal inflammation, which is becoming increasingly prevalent in Western societies, and is spreading to the rest of the world. Although the etiology of IBD is poorly understood, it is widely accepted that several factors may be crucial, which includes genetics, diet and lifestyle, immunity, environment, and microbiota. In the past decade, huge advances have been made toward a better understanding of IBD, epitomized by far-reaching progress in the field of microbiome. In this review, we summarize the current knowledge of how changes in microbiota may affect the pathogenesis of IBD. Commensal bacteria, benign normally, are essentially opportunists that may readily take over, and potentially contributes to dysbiosis, which, in turn, promotes pathogenesis. Several pathogens, mainly *Mycobacterium avium paratuberculosis*, adherent invasive *Escherichia coli*, *Clostridium difficile*, *Campylobacter*, and *Salmonella*, have been shown to be associated with IBD, but the causality remains unproven. The microbiome comprises of not only bacteria, but also viruses, bacteriophages, and fungi. However, little is known as per the role of the latter. We highlight recent research on viruses, bacteriophages, and fungi in IBD. We also discuss the progress on manipulating the microflora to serve therapeutic purposes. The methodology for manipulating the microbiota covers fecal transplantation, pre-, pro-, syn- and post-biotics, helminth therapy, bacteriocins, bacteriophage, etc., which greatly enriches the arsenal against IBD. Manipulation of intestinal microbiome represents a promising type of therapy for IBD.

**Keywords:** Bacteriotherapy; Butyrate-producing bacteria; Dysbiosis; Fecal microbiota transplantation (FMT); Fungi; Inflammation; IBD; Lactic acid bacteria; Lactobacillus; Fructooligosaccharides; Helminth therapy; Microbiome; Microbiota; Metagenomics; Pathogenic bacteria; Probiotics; Virus

## Introduction

Characterized by redness, tissue swelling, feelings of heat and pain, and loss of function, inflammation is essentially the body's foremost response against detrimental stimuli such as trauma, laceration, infections, irritants, allergy, frostbite, heat shock, hypoxia, etc.[1], which constitutes a critical part of innate immunity. Inflammation is categorized into acute inflammation and chronic inflammation. During acute inflammation, driven by a few signaling cascades, dilation of arterioles increases localized blood flow. Fluid, electrolytes and other blood components accumulate in the interstitium due to permeabilization of the microvasculature. Neutrophils, and possibly some macrophages, infiltrate into the site of injury, which is implemented by the interactions of cell adhesion molecules (CAMs) including selectins and integrins. The duration varies from seconds or minutes, to a few days. If the stimulating agent is not eliminated in the stage of acute inflammation, which is possible upon cellular stress, microbial infection, epithelial barrier malfunction and suboptimal environment, the inflammatory episode proceeds and develops into distinctive conditions, and that is when chronic inflammation occurs [2,3]. Upon injuries mast cells, damaged endothelium, neural synapses, platelets and neighboring cells release cytokines and chemokines to recruit neutrophils, which, together with macrophages, produce TNF-α, IL-1β, IL-6, IL-8, IFNγ, prostaglandins, etc. This triggers the activation of NF-κB, JAK-STAT, p38-MAPK, PI3K-AKT, β-catenin, and JNK pathways [4]. The pathogens are then disposed of by mobilized T-cells and B-cells, accompanied by the release of reactive oxygen species (ROS) and destructive enzymes.

Intestinal inflammation, while retaining all the common features of the aforementioned inflammatory repertoire, displays distinctive pathological landscape due to the specificity of the organ. It may be initiated by physical injuries, viral invasion, bacterial infection, genetic defects, etc. Inflammatory bowel disease (IBD) is an intestinal disorder featured by intermittent outbreaks of inflammatory destruction. There are two major types of IBD, ulcerative colitis (UC) and Crohn's disease (CD). UC is manifested exclusively in colonic mucosa, whereas CD shows symptoms such as granulomas and intestinal fibrosis throughout all areas of the gastrointestinal (GI) tract [5]. The highest incidence of IBD is in North America and Europe. In the US alone, an estimate of 1.4 million patients is diagnosed as IBD, and rates continue to climb in some other countries in Asia, northern Africa and Oceania [6]. It is generally believed that genetics, immunity, environmental factors, and microbiome all contribute to the pathogenesis of IBD [7]. This review will discuss microbiome and IBD. We summarize the research progress of host-microbe interactions in IBD and illustrate how to manipulate the gut microflora to serve therapeutic purposes.

## Gut Microflora and its Physiological Functions

### Shape the gut flora

Co-evolution has forged a symbiont relationship between host and microbiota through millions of years, thereby imposing a delicate balance between immune activation and immune tolerance on the gut [8,9]. As reviewed by O'Hara AM et al., the intestine is adapted to bi-directional host-flora exchange and harbors a diverse bacterial community that is separated from the internal milieu by only a single layer of epithelial cells [10]. The resident microflora has a collective metabolic activity equal to a virtual organ within an organ [10]. While gut flora mainly live within the intestinal lumen, a tiny portion of the gut microflora populates the crypts [11].

Bacteria that populate the gut are 10 times greater in sheer number than the eukaryotic human cells, in parallel with a 100 times larger gene pool than the human genome [12]. Intestinal microbes have a luminal concentration of near 0 in stomach to as high as $10^{11}$ to $10^{12}$ per gram in colon [13]. Mammals get the gut flora established by maternal transmission from birth canal and breast feeding. It is believed that bacterial community is also inherited in the womb [14], with the delivery mode, vaginal or cesarean-section (C-section), conferring distinctive bacterial communities on the neonates. While infants delivered vaginally generally possess microbiome similar to the vaginal microbiome, C-section babies harbor microbiota resembling that of skin surface [15]. The bacterial community is then shaped by early exposure to environment. The contribution of environmental factors, was further corroborated by mouse studies [16]. Turnbaugh et al., conducted a research in lean and obese twins with metagenomic analysis tools [17]. The study involves female monozygotic and dizygotic twin pairs and their mothers. They showed that the twin-versus-mother co-variation is comparable to that between both monozygotic and dizygotic twins at the gene level of gut microbiome. Thus, they concluded that gut microbiome is shared among family members, but that each person's gut microbial community varies in the specific bacterial lineages present, with a comparable degree of co-variation between adult monozygotic and dizygotic twin pairs [17]. The composition of gut flora is in a sporadic form in infants and eventually undertakes a globally homeostatic composition in adults if there is no therapeutic or accidental intervention [18]. Taken together, these studies suggest that genetic background and environment factors all contribute to the profile of microbiome.

### Physiological functions of microbiome

The corporate number of microbial species in human gut is estimated to be 1000 to 1150, with each individual harboring at least 160 [19]. A large portion (38%) of the total gene pool is commonly shared from individual to individual. The "core human microbiome" refers to the central part of microbial gene pool existing in all or most of humans. Now it is increasingly clear that the interindividual similarity is only present at gene and functional level, but not in terms of organismal lineage [16]. The "variable human microbiome" is the microbial genes in a specific cohort of people, which is based on a combination of host factors [20].

To profile the microbiome constituency of human, the "supraorganism", Human Microbiome Project (HMP) and Metagenomics of the Human Intestinal Tract (MetaHIT) Project were launched [21]. Comparative metagenomics unveiled a wide variety of functions fulfilled by gut microbiota which include: i) gleaning indigestible ingredients from food and synthesizing nutritional factors such as vitamins; ii) detoxifying the deleterious xenobiotics, and affecting the host metabotypes; iii) providing signals for epithelial renewal, maintaining gut integrity; iv) replacement of pathogenic bacteria by colonization resistance; v) secreting anti-microbial products, e.g. bacteriocins and lactic acid; vi) development of a robust systemic and intestinal immune system that is driven by a well-developed gut microbial community; and vii) determining various physiological states such as cardiac size, and behavioral patterns [10,20].

*Firmicutes* and *Bacteroidetes* predominate the gut microbiota, followed by *Proteobacteria* and *Actinobacteria*, with minor contributors including *Verrucomicrobia* and *Fusobacteria* [22]. *Bacteroides and Ruminococcus* are consistent with enriched intake of animal sources, while a plant-based diet favors *Prevotella* [23]. *Prevotella* to *Bacteroides* ratio constitutes an important index for clinical diagnosis. Butyrate-producing bacteria, including *Clostridium* groups IV (*Faecalibacterium prausnitzii*) and XIVa, *Roseburia spp.*, *Butyricicoccus*, and lactic acid bacteria (LAB), mainly *Lactobacillus* and *Bifidobacterium*, are believed to benefit the host through anti-inflammation, anti-tumorigenesis, and pathogen exclusion [24-26]. There is also a metabolic interplay between LAB and butyrate-producing bacteria due to the ability of the latter to feed on lactate [27].

In summary, gut flora is shaped gradually during the early stage of life and stays *status quo* in adulthood without dramatic intervention. The physiological repertoire it fulfills involves digestion, nutritional supplementation, detoxification, educating the immune system, behavioral modulation, etc.

## Microbiome in IBD

### Commensal bacteria and dysbiosis in IBD

Commensal bacteria are mostly opportunistic rather than completely non-pathogenic, which means that they may contribute to the pathogenesis of IBD. $IL10^{-/-}$ mice appear healthy under germ-free (GF) conditions, but quickly develop colitis in a specific-pathogen-free (SPF) environment [28]. In axenic $IL10^{-/-}$ mice, sterile bacterial lysates compromise the integrity of the intestinal barrier, but fail to initiate sustained inflammation [29]. *Candidatus arthromitis*, a gram-positive and unculturable species in *Clostridium*, also known as segmented filamentous bacteria (SFB), commonly adheres to intestinal epithelium, spurs potent immune responses in gut and is under the tight control of IgA [30-33]. As a commensal microbe, SFB has an immune modulatory role in driving Th17 cell maturation, and even Th1 and T regulatory (Treg) responses and promotes the maturation of host mucosal barrier [30,34,35]. The colonization of SFB enhances inflammatory and anti-microbial actions, and improves the resistance against pathogenic bacteria like *Citrobacter rodentium* in mice [36]. On the other hand, its proinflammatory properties contributes to IBD in SCID mice reconstituted with CD4(+) T cells from healthy BALB/c mice, as manifested by diagnostic indices [37]. *Bacteroides fragilis* participates in the induction of active mucosal tolerance by producing polysaccharide A (PSA), which stimulates CD4(+) T cells to trans-differentiate into Foxp3(+) Treg cells [38]. *Bacteroides infantis* and certain blend of *Clostridium* groups IV and XIVa can lead to comparable responses [39]. *Butyricicoccus pullicaecorum* has a protective effect against 2,4,6-trinitrobenzenesulfonic acid (TNBS) - induced colitis [40]. Supernatant from its culture prevents the loss of

epithelial integrity in Caco-2 cell line [41]. Administration of *F. prausnitzii* induces Treg activation, elevates anti-inflammatory cytokines, strengthens intestinal barrier, and reduces the severity in several colitis models and helps maintain clinical remission in UC patients [42-45]. Lactic acid bacteria (LAB) benefit the hosts in a similar manner [46-50]. It should be noted that several animal models of IBD may not only be dependent on the microbiota. For example, dextran sulfate sodium (DSS) induced colitis is routinely used for IBD research as a chemical-induced model [51].

Human IBD occurs when there is an unfortunate combination of dysbiosis and genetic susceptibility. Dysbiosis is a perturbed condition of microbiota, which manifests from a weakened capacity to counterbalance bacterial constituency and also to withstand the environmental and host brunt. The data support the notion that it is very much accountable for the ever increasing IBD occurrence [52-55]. In IBD, the diversity of microbiome is reduced. Metagenomics revealed a 25% reduction in genes harbored by the disease-afflicted patients [19]. Some studies show that *Mycobacterium avium paratuberculosis* (MAP), *Clostridium difficile*, *Ruminococcus gnavus*, and adherent invasive *Escherichia coli* (AIEC) are enriched, while *F. prausnitzii*, *Roseburia hominis*, *Lactobacillus*, *Bifidobacterium*, and *Akkermansia*, which is mucin-attachment dependent, are depleted in IBD patients [56,57].

## Pathogenic bacteria associated with IBD

There is no conclusion whether there are indeed some pathogens playing a causal role in IBD. The most controversial candidates underlying the etiology are *Mycobacterium avium paratuberculosis* (MAP), adherent invasive *Escherichia coli* (AIEC), *C. difficile*, *Campylobacter*, and *Salmonella*. Although therapeutic trials directed against potential causative infectious agents have been unsuccessful to date, it is possible that the studied IBD patients were a heterogenous population with multiple etiologies for their IBD. Therefore, a potential benefit could not be demonstrated in the subgroups of patients where one organism may have been driving their IBD.

MAP is widespread in drinking water, milk and other dairy products, as well as meat, and is able to survive pasteurization [58,59]. In ruminants, e.g. cattle, MAP infection culminates in Johne's disease, a granulomatous type of enteritis that resembles human CD [60]. It severely infects intestinal goblet cells, eliciting epithelial damage and inflammation [61]. The presence can be detected in breast milk, blood, intestinal mucosa, and gut biopsies of CD patients [62-65].

Controversies arise as per its role in IBD. MAP-positive incidence appears non-significantly higher in the feces of CD patients than in those of healthy controls [66]. Furthermore, the positive incidence is significantly higher in biopsies of terminal ileum and colon in CD patients than in those from healthy controls [67]. However, counteracting studies disproved such association [68-71]. Anti-MAP treatment with antibiotics does show short-term benefits in enhancing corticoid-induced remission, but hardly sustains the improvement over a longer course (156 weeks) [72]. MAP/self-cross-reactivity can be detected in CD patients, but not in healthy controls, imposing higher risks of autoimmunity attack, which implies a correlation between MAP and CD at most notwithstanding [73]. There are studies involving UC as well, but the link with MAP is still missing.

AIEC attaches itself to intestinal mucosa with type I pili and long polar fimbriae (LPF) [74,75]. It is structurally adapted to withstand insult from and replicate within in vacuoles of macrophages [76,77]. By promoting TNF-α release from macrophages, or direct interactions with enterocytes, AIEC stimulates enhanced expression of CEACAM6, a molecule for binding of the type I pili, resulting in more AIEC attachment [74]. AIEC inhibits autophagy by triggering NF-κB signaling and subsequent reduction in ATG5 and ATG16L1, eliciting excessive inflammation [78]. Biopsies from CD patients support the implication of AIEC by increased prevalence, abundance, as well as richness, which, however, is not a proof of causation [79].

*Clostridium difficile* infection (CDI) has doubled in occurrence during the period from 1996 to 2003 and is still steadily expanding in the following years in western countries with the development of antibiotic resistance [80-82]. CDI may lead to colectomy and lethality, and has become more severe due to the emergence of B1/NAP1/027, a more virulent strain characterized by enhanced fluoroquinolone resistance, and higher levels of toxins A and B, and binary toxin [83]. The recurrence rate may be as high as 25% within 30 days after metronidazole or vancomycin treatment [84,85]. The symptomatic presentations of CDI and IBD are hardly distinguishable (diarrhea, leukocytosis, hypoalbuminemia, and fever) [86,87], and so poses difficulty for pinpoint therapy. Evidence is accumulating on the linking of CDI with IBD flares and relapses, as indicated by 5% to 60% toxin positive rates in the latter [86]. Incidence of CDI is 2.9-, 4.0- , and 2.1-fold of non-IBD controls for IBD, UC and CD patients respectively [88]. Some immunosuppressive drugs administered for IBD treatment double or triple the incidence of CDI (thiopurines, methotrexate, steroids, corticosteroids), while others, e.g. infliximab, do not show such effects (corticosteroids) [89]. Risk factors for CDI in IBD also involve age, antibiotics, hospitalization, etc. [87]. Recently, region-specific and highly sensitive assays cast questions over the prevalence of CDI in IBD, indicating that the correlation may be confined within a subset of patients [90,91]. It remains unclear whether CDI contributes to IBD inflammation or whether IBD patients are just more susceptible to CDI resulting in a secondary inflammatory process related to the infection.

The prevalence of *Campylobacter* species, particularly *C. concisus* and *C. ureolyticus*, is significantly higher in IBD patients than in non-IBD controls [92-96]. The correlation may rest on the microbes that reside in the GI tract. There exists an active interplay between gut flora and *C. concisus* in CD [97]. *C. jejuni* can possibly ruin the intestinal barrier by translocating commensal bacteria, priming chronic inflammation [98,99]. It is easy to confound *Campylobacter jejuni* enterocolitis with IBD, judging from the clinical presentations [100,101].

Infectious triggers are implicated in the onset and reactivation of IBD by epidemiologic and clinical studies. Exposure to non-typhoid *Salmonella* significantly increases the risk for both CD and UC in the first year, followed by a gradually reducing tendency of IBD flare in a 10-year time frame with a progressively narrowing difference compared with non-infected controls [102]. The same study revealed a risk curve correlated with *Campylobacter* for UC that extremely resembles that of *Salmonella*, therefore favoring a non-causal role for both bacteria. Mark et al. argued that the study is ambiguous due to the detection bias, and a failure to take into account the effects of infection on IBD in susceptible population [103]. The notion that *Salmonella* and *Campylobacter* infection predisposes patients to IBD onset is consistent with some other studies linking the infection with IBD pathogenesis [104,105]. However, CD patients exposed to *Salmonella enterica* do not differ in major histological indexes and necessity for various types of treatment from drug administration to

surgery compared with patients not exposed to *Salmonella*, indicating a lack of correlation between the infection and CD especially in terms of severity [106]. Besides, enterotoxigenic *Bacteroides fragilis*, *Listeria monocytogenes*, *Aeromonas hydrophila*, *Chlamydia sp.* are enlisted in the bacterial troops associated with IBD as well [87].

Overall, whether pathogenic infection is a sequel or causality of IBD is a topic of ongoing debate. Endeavors on deciphering this complication wind up in 3 hypotheses: i) there is a subset of gut flora that not only triggers, but also persists after the onset of IBD, however, the identification exceeds the capacity of current methodology; ii) the causative agent has been wiped out by the time of disease occurrence; iii) the microbiome is influenced by underlying defects in the mucosal immune system and does not directly cause the inflammation [107].

### Viruses, bacteriophages and fungi in IBD

Easily neglected is that the microbiome comprises of not only bacteria, but also viruses, bacteriophages and fungi. In a sharp contrast, the role of the latter in IBD is poorly defined. These organisms are difficult to isolate and study in the context of intestinal inflammation and so modern techniques will have to evolve to study this critical part of the microbiome.

Epstein-Barr virus (EBV) is commonly detected in the GI mucosa with inflammation [108]. It is significantly more prevalent in intestinal tissues of IBD patients than in healthy controls, and more frequently detected in patients with exacerbation than those in remission [109]. Furthermore, EBV may prolong the inflammation in IBD and enhance replication in B-lymphocytes [110]. Intestinal infection of cytomegalovirus (CMV) may contribute to the severity of UC [111], but again it is not clear whether CMV is contributing to IBD inflammation or is acting as an innocent bystander. Patients with IBD have a long-lasting remission and reduced relapse rates with HIV infection, which is possibly related to depletion of CD4 T cells [112,113]. Measles virus was believed to trigger CD upon specific manners of exposure [114,115], which was supported by some researchers, but the persistent infection theory was disproved by PCR, serology and molecular mimicry [116-119]. Infection with murine norovirus (MNV) in mice with ATG16L1 mutation leads to defects in Paneth cells and exacerbated pathological changes induced by DSS which resemble those seen in CD [120]. Bacteriophages, predominantly *Caudovirales* phage, exist in large abundance in gut wash and ileal biopsies of CD patients [121]. The density in gut mucosa may reach 10/mm$^3$, the composition of which substantially underlies dysbiosis and immune responses in IBD [122]. Fungi in gut mostly consist of ascomycetes including *Candida, Penicillium*, *Saccharomyces* genera, and various species of basidiomycetes, the diversity of which increases in IBD [123]. *Candida*, particularly *C. albicans*, is well adapted to and commonly colonizes all sections of GI tract [124]. The familial CD is shown to be associated with a higher prevalence and abundance of *C. albicans* [125,126]. Anti-*Saccharomyces cerevisiae* antibodies (ASCAs) which target a conserved cell wall epitope of fungi including *C. albians* and *S. cerevisiae* have been associated with manifestations of CD [127,128]. The prevalence of *C. albicans* in colonic mucosa is higher in UC patients than in normal controls, therefore the positive serological reactivity can serve as a diagnostic marker [129]. Some studies showed that *Candida* slows colonic ulcer healing, which could be reversed by anti-fungal treatment, but, again, the causality in IBD remains to be proven [130-132]. Studies of viruses, bacteriophages, and fungi in the cause and progression of IBD are needed.

## Clinical Therapies for IBD Through Manipulating Gut Flora

Most care for IBD occurs in the outpatient setting, with hospitalizations reserved for complications including surgery. Traditional treatment of IBD adopts the strategies of anti-inflammation, dietary management, and surgery, and mitigates the symptoms to a certain extent [133]. Despite recent advances in therapy, there continues to be a demand for highly effective and safe therapies for IBD patients. The manipulation of microbiota has the potential to be a therapeutic strategy for IBD. There are various approaches to target the gut flora, including fecal microbiota transplantation (FMT), pre-, pro-, syn- and postbiotics, parasitic worms, diet, etc. [134-136]

### Fecal microbiota transplantation

FMT was first recorded in "Zhou Hou Bei Ji Fang", a medical manual for emergencies, written in 4th-century China, by Ge Hong, a herbal medicine master and alchemist, who successfully saved lives from food poisoning and diarrhea by oral administration of fecal suspension [137]. The therapeutic potential became the public gaze in modern medicine in the early 1900s, followed by a cohort of modern case reports and case series that have revealed significant cure rates [12,138-141]. The notion of FMT is that the normal microbiota in gut defends the GI mucosa against virulent bacteria through competitive exclusion which is called "barrier effect", and also fermenting and secreting unused energy substrates such as butyrate to train immune system and prevent growth of harmful, pathogenic bacteria [142]. Fecal suspension is prepared with saline, milk or yogurt, and delivered to the patients by nasogastic/nasoduodenal intubation, enema or colonoscopy [137,140]. Meticulous donor screening may alleviate many of the concerns of transmitting infections including HIV, viral hepatitis, CMV, EBV, etc. [140,143].

FMT represents a relatively safe and efficacious method for the treatment of IBD and CDI in clinical practice, but requires additional study. About 92% patients with CDI reported has alleviated or resolved the symptoms after FMT with low incidence of relapses [144]. There are cases reported showing that 76% IBD patients experience overall improvement, with others getting no resolution of the symptoms [144]. However, the effectiveness of FMT in UC and CD is not convincing until therapeutic effects are observed with a larger cohort and longer follow-up. Protocols towards higher resolution are being developed, e.g. serial FMT. Few serious direct or related adverse effects were observed in the operation of FMT although fewer than 10% of patients after FMT reported flatulence, diarrhea, fever, blood stool, etc., which were tolerable and self-limiting [145]. The small number of adverse events that have been reported following FMT procedures can not necessarily be linked to the procedure or transplant itself [146,147]. FMT procedure may not be advised in patients with significant intestinal inflammation.

In clinical practice, fresh stool can be refined by mixing with buffer solution and filtering. The preparation, while containing the full spectrum of microbiota, repeals the offensive odor of the feces. Also, it is supposed to harbor the standardized gut microbiota that a healthy individual carries. The homogenate can be delivered to patients in order to restore the disturbed bacterial community, and also, cryopreserved for long-term use [148].

Clinicians may be able to select bacterial species of interest to get a defined mixture. This method, called "blended bacterial culture",

dispenses the need to find a standardized fecal sample, and has a manageable formula. In treated patients, fecal bacteriotherapy using bacterial culture showed some therapeutic effects, reestablished the normal gut pattern over the short term and maintained remission for 6 months [149]. However, this method is not a typical clinical procedure and only limited patients were tested. The abilities of the bacterial culture to populate the gut and to cure IBD among larger groups of patients are still open to criticism.

## Prebiotics, probiotics, synbiotics and postbiotics

Prebiotics represent a group of carbohydrates that cannot be degraded by the host but can otherwise promote the growth and activity of beneficial bacteria within the GI tract. Probiotics are microbes that bestow the host advantages in maintaining microbiota homeostasis given enough dosage. Probiotic bacteria were originally based on lactic acid bacteria (LAB), and then extended to *Bifidobacterium*, *Escherichia coli* Nissle 1917, and *Saccharomyces boulardii* [150]. They compete against pathogens by taking over the space and sites of binding and rendering the environment unfavorable by lowering the pH, and secreting bacteroicins and chemicals toxic to the harmful bacteria [25,151,152]. Synbiotics stand for a combinatory use of prebiotics and probiotics if there exists a synergistic benefit, while postbiotics are metabolic products of the probiotics, promoting homeostatic growth of the microbiota.

Some prebiotics are oligosaccharides such as fructooligosaccharides (FOS), xylooligosaccharides (XOS), galactooligosaccharides (GOS), lactosucrose, and polydextrose. Others are plant-based ingredients like inulin, bran, pectin, and konjac mannan. Researchers now focus on the distinction between short-chain, long-chain and full-spectrum prebiotics and find that different prebiotics are fermented in different parts of intestine and nourish bacteria in local areas. Prebiotics are reported to work on immune responses besides improving digestion and absorption. Infants fed with formula enriched in prebiotic GOS/FOS showed enforced production of fecal sIgA [153]. Cell signaling studies suggest that probiotics may interfere with NF-κB signaling, interact with TLRs, and modulate inflammation by enhancing secretion of IL-10, increasing $Foxp3^+$ Treg cell population, and suppressing production of inflammatory cytokines including IFN-γ, TNF-α, IL-8 and IL-12 [154,155]. *S. boulardii* administration is effective in reducing CDI recurrence, while *Lactobacillus* plus metronidazole treatment turns out to be unhelpful [86]. VSL#3 is a commercial blend of eight probiotic bacteria, including four *Lactobacillus species*, three *Bifidobacterium* species and *Streptococcus thermophilus* [156]. Although *E. coli* Nissle 1917 and VSL#3 show clinical benefits in the maintenance of UC and pouchitis, the administration seems ineffective in CD [157]. Recent mechanistic studies suggest prebiotics are beneficial to CD patients through production of short chain fatty acids (SCFA) to nourish the colonic walls, and beneficial to UC through reduction of hydrogen sulfide gas due to reduction of sulfate-producing bacteria, as food supplements specifically enhance the growth of SCFA producing bacteria such as *Clostridia* and *Bacteroides* in intestine, and it has been clearly demonstrated that prebiotics lead to increased production of the SCFAs [158].

## Helminth therapy

According to the "IBD hygiene hypothesis", IBD is caused by exceedingly vigorous immunity against intestinal contents, and people raised with extreme hygiene may be impaired in immune development and have higher risks of IBD in later life [159]. In developed industrialized countries, well-constructed hygienic protocols have wiped out intestinal helminthic parasites [136]. In order to establish chronic colonization, parasitic worms evolved potent mechanisms to efficiently regulate host immunity, especially via quelling inflammation [160]. Exposure to *Schistosoma mansoni* or *Hymenolepis diminuta*, either live or in the form of extracts, have protective effects against TNBS-induced colitis [136,161]. In clinical trials, *Trichuris suis* (pig whipworm) and *Necator americanus* (human hookworm) hold some potential in IBD treatment [162,163]. One potential benefit from helminth therapy may be due to a switch of the microbiota. Infection with *Heligmosomoides polygyrus bakeri*, together with an increase in total bacterial load, the *Lactobacillaceae* family was significantly enriched in ileum, which is mostly lactic-acid producing bacteria including *Lactobacillus* [135]. The dramatic shift may be either caused directly by helminths or by altered immune responses from the host. However, a recent phase II study indicated that the *Trichuris suis* treatment for IBD lacked efficacy. [http://www.medpagetoday.com/Gastroenterology/InflammatoryBowelDisease/42805].

## Other approaches to manipulate enteric flora

Species- and strain- specific vaccines have been developed, targeting Enterotoxigenic *E. Coli* (ETEC), uropathogenic *E.coli*, recurrent *C. difficile* infection (R-CDI), etc. [142]. Unlike traditional antibiotic therapies, which kill most of the microrbiota in gut, or novel therapies like FMT, which provides full-spectrum microbiota of stool, bacterial vaccines lead to clearance of IBD associated pathogenic bacteria specifically. As mentioned earlier, there is no evidence that these organisms are causative. Thus, it is unclear how effective vaccines will be for the treatment of IBD. Bacteriocins, anti-microbial peptides produced by bacteria, are widely used as a food additive to intervene gut microbiota by reducing pathogenic microbial population [164,165]. Bacteriophage therapy gradually becomes enticing due to ever exacerbating drug resistance [166].

Genetically engineered bacteria have been developed to produce cytokines and growth factors to help repair tissue damage. The pioneering work of Steidler and colleagues introduced genetically engineered *Lactococcus lactis* that produces IL-10 to DSS-treated mice, reducing the incidence of colitis by 50% [167]. Another study using weakened *Salmonella Typhimurium* SL7207 carrying superoxide dismutase and anti-inflammatory peptides showed some benefits in a murine colitis model [168]. Along with this line, Hamady and colleagues constructed a stain of *Bacteriodes ovatus*, an anaerobe that adheres to the mucus layer, to secrete KGF2 [169]. The gene was placed downstream of the xylanase promoter so the factor is produced only when xylan is administered. The treatment restored epithelial integrity and mitigated the symptoms of colitis. By tropical delivery, the therapeutic dose could be reduced to 0.01-0.1% of that needed for systemic administration [11]. However, there is a gap to transform bench findings to clinical practice.

In summary, manipulation of intestinal microbiome represents a promising type of therapy for IBD, which may lead to long-lasting remission for patients and provides an alternative therapeutic approach. The methodology covers fecal transplantation, pre-, pro-, syn- and post-biotics, helminth therapy, bacteriocins, bacteriophage, etc., which greatly enriches the arsenal against IBD. However, the promise of pharmabiotics is unlikely to be completely fulfilled without a greater understanding of enteric microflora. Elucidating the

molecular details of host-flora interactions is, therefore, a pre-requisite for a "bugs to drugs" program of discovery.

## Future Prospect

*Dao De Jing*, the foundational document of Taoism written by Lao Zi in 500s B.C., once wisely put it, "Everything carries Yin and embraces Yang. In clashing, the two poles of qi unite." As per traditional Chinese medicine theory, disease, by nature, is an imbalance of Yin and Yang that drive human bodies, therefore the conditions can be categorized into i) Yin excess; ii) Yang excess; iii) overabundant Yin leading to insufficient Yang; iv) overabundant Yang leading to insufficient Yin; v) dual deficiency of Yin and Yang; vi) dual excess of Yin and Yang.

The thoughts well apply to the roles of microbiota in intestinal homeostasis. Unity of opposites constitutes the core rule that arbitrates the fate of the gut. It runs the risks of simplification to group the bacterial community into beneficial and detrimental, albeit it seems so under controlled scenarios. Philosophically speaking, counterbalancing opposites transform mutually provided proper causes. The current success of therapies on IBD rely heavily on the subliminal practice of promoting balanced gut flora, thereby seducing people into a metaphysical perspective that we can define what's "good" and by consuming a lot of the "good" intestinal disorders can be cured, which, however, gradually deviates researchers from the right track. Communicated by the sayings of Lao Zi, "Yin" and "Yang" do not equal to negative and positive as they literally mean, but rather intermingled opposites that present themselves as the best when the other is rising with comparable momentum. That is why probiotics usually label the range of daily dosage on the bottles. In agreement with the majority of peers in GI field, we favor FMT the most, since in all the available therapeutics, it to a great extent restores the interplay among bacterial populations.

It is less likely to come up with more effective measures to reestablish gut homeostasis without an accurate description of the functions of each bacterial group and their relationships with the host and environmental factors. We propose that the function-based grouping is more meaningful than merely taxonomic classification, which is consistent with the superiority of empirical models in clinical trials. It's based on the same concept that the interindividual similarity is manifested at gene and functional level, accounting for the resilient and refractory nature of microbiome in response to environmental stresses. Diet, lifestyle, innate and adaptive immunity, and the environment are manipulable leverage to rectify the tilted microbial balance.

Genetic defects lead to a dwindling capacity of the hosts to contain the otherwise unharmful bacteria from turmoil and pose potent threat to gut robustness. There are more than 163 confirmed genetic risk loci in IBD including the more and more definitive NOD2, ATG16L1, and IL23R, with 110 shared between CD and UC, and most of them display physiological relevance with epithelial barrier, interactions and responses towards gut flora, autophagy, and maladaptive Th17 type adaptive immunity [170,171]. Studies have attempted to address the functions of these genetic loci in shaping intestinal microbiota, and perhaps the specificity of microbial-risk loci association will be the next hot topic in the studies and treatment of IBD. Researchers also attempt to link inflammatory activity with intestinal microbial biomarkers of inflammation, such as serum C-reactive protein levels and fecal calprotectin. The correlation of microbial biomarkers and inflammatory activity is also critical to the choice of treatment strategy and to monitor treatment efficacy in clinical trials.

Additionally, recent studies mainly focus on bacteria in gut microbiota. However, fungal microbiota and how its metabolites impact GI function and contribute to the pathogenesis of IBD are ignored, as well as the effects of viruses, archaea, and phages. Metagenomic analyses of intestinal microbiota suggested such microbes were also required for the development of IBD as an overall increase in fungal diversity was observed in IBD patients. The relationship between these organisms and IBD will no doubt be explored in more detail in the coming years.

With a similar point of view we use to dissect the bacterial community, the essence of the intestinal homeostasis is not only based on the host or the microbiome, but also the interactions and coexistence of them both. Further elucidation of the correlation between intestinal microbiota and IBD may enable the design of artificial stool with a clear formula and an individualized menu for people with a specific genetic background. Manipulation of microbiome suits the concept of counterbalancing, and would show continuous therapeutic promise in future trials.

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