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Review Article

Microbiota and Gut Health: Promising Prospects for Clinical Trials from Bench to Bedside

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Inflammatory bowel disease (IBD) is a chronic inflammatory gastrointestinal disease characterized by weight loss, abdominal pain, and bloody diarrhea. The number of affected patients has increased in recent years. Despite the fact that scientists have been studying the pathogenesis of IBD for many years, the specific pathogenesis pathway remains unclear. As a result, none of the therapeutic approaches can cure IBD patients completely. However, the increasing research factors associated with the incidence of IBD are reasonable. These variables can be divided into two categories: microbiome-related factors (bacteria, fungi, and viruses) and nonmicrobiome-related factors (diet, gene, host immune system, gender, and ethnicity). Surprisingly, we found that all the variables impact the gut flora in IBD patients, either directly or indirectly. Dysbiosis of the gut microbiota eventually leads to an increase in the incidence of IBD. As a result, therapeutic targets focusing on correcting dysbiosis in the gut microbiome, including using probiotics and postbiotics, could become one of the most promising IBD treatments in the future. We went through each linked factor and explained how they contribute to an increased risk of IBD. We will review some existing conventional therapies for IBD before moving on to a revolutionary therapy strategy that employs prebiotics, probiotics, and postbiotics to treat IBD based on the criteria stated. Furthermore, different persons have varying reactions to the same probiotic strain. As a result, we also provide the option of having individualized probiotic medication tailored to each IBD patient.

1. Background

Inflammatory bowel disease (IBD) is a chronic inflammatory gastrointestinal disease, including ulcerative colitis (UC) and Crohn's disease (CD) as two subtypes, usually with symptoms such as weight loss, abdominal pain, and bloody diarrhea [1]. In the past decades, the number of affected people has increased worldwide among the population with age between 15 and 40s [2]. Currently, IBD does not have effective treatments in clinical practice, as all the medical treatments focus on relieving the pain and ameliorating the inflammation in the gut, such as aminosalicylates or mesalamine to reduce inflammation, immunosuppressants to reduce the activity of the immune system, or antibody-based treatments [1]. In additional to drug-based treatment, if patients develop severe symptoms that cannot be alleviated by medicines, surgery will

be performed to remove the inflamed colon [3]. If the IBD is not treated promptly, a proportion of patients might progress into an advanced pathogenic stage, such as colorectal cancer. Because of the recurrent and unpleasant symptoms of IBD, patients might need regular medical treatment for the rest of their lives. As a result, an effective treatment to reduce the recurrence of IBD is urgently needed.

The causes of IBD remain unknown. Previous studies have suggested that IBD might be caused by a combination of factors, including but not limited to genetics, dietary factors, and dysbiosis of the gut flora [4]. Diet and its association to IBD have long been investigated. According to the existing research findings, the influence of dietary factors on IBD etiology is a bit of contradictory. High sugar and animal fat consumption is usually associated with an increased risk of developing IBD. On the other hand, a meal which contains more citrus fruit and rich fiber might protect against IBD [5]. Meanwhile, the genome-wide association study (GWAS) provides a plethora of evidence of how gene expression affects the etiology of IBD. This study revealed 12 essential driver genes that are identified to play a critical role in modulating the regulatory network state in IBD [6]. Other than dietary factors and host gene, recent studies have demonstrated that the dysbiosis of the gut microbiota is intimately linked to IBD. One study comparing the microbiota composition of IBD patients to the healthy individuals found that they have different composition, abundance, and structure of bacteria. For instance, the number of *Christensenellaceae*, *Coriobacteriaceae*, and *Faecalibacterium prausnitzii* decreased in IBD patients' fecal samples, while the abundance of *Actinomyces*, *Veillonella*, and *Escherichia coli* increased in IBD patients' fecal samples [7]. Therefore, modulation of the microbial environment could be a therapeutic approach to treat IBD. As a result, in hospitals, probiotics are given to patients accompanied by anti-inflammation and pain-releasing drugs to help correct the dysbiosis of gut microbiota for IBD patients. The effects of probiotic treatment had a substantial impact. A recent study showed that probiotics could rebuild the patients' microbiome and improve the disease phenotype [8].

Moreover, probiotics' metabolites, also named postbiotics, can restore the gut microenvironment with its effect mainly in the small intestine rather than the colon [9]. *Lactobacilli* spp. and *Bifidobacterium spp*. are the most frequently used microbiota in probiotics [10]. *Lactobacilli spp*. functions in boosting the reconstruction of the gut microbiome of patients and downregulating the inflammatory cytokines and chemokines [11]. *Bifidobacterium spp*. are shown to have effects on reducing levels of critical IBD-related proinflammatory cytokines such as TNF-α, IFN-γ, and IL-1β and increasing the production of IBD protective cytokines TGFβ and IL-10 in vivo [12]. As a result, most of the probiotics used in clinical treatment comprised these two species. However, symptoms vary depending on the stage of IBD, and different types of IBD affect various parts of the gastrointestinal tract. To address this in detail, most patients in the early stages of Crohn's disease (CD) experience diarrhea or abdominal pain. Moving to the severe location, fever or anemia will worsen the situation [13]. UC mainly occurs in the large intestine, whereas CD typically occurs in the small intestine. Customized medicines with various combinations of bacterial taxa at the different pathogenic phases and inflamed areas in the gut have not yet been proposed for IBD patients. To address this gap, the present review covers the contribution and potential interaction between internal and external factors, including dietary factors, host gene, immune system, and microbiota. Based on recent research findings, we also outline various probiotic combinations in clinical trials, allowing patients to pick which to take according to their stages and types of IBD.

2. Factors Associated with the Pathogenesis of Inflammatory Bowel Disease (IBD)

2.1. Dietary Factors as a Nonmicrobiome-Related Factor. Dietary habits, as one of the significant risk factors of IBD, have been well studied for many years. According to research findings, people with high animal fat, sugar, and alcohol are more likely to develop IBD than those with a regularly vegetarian fiber intake and low alcohol consumption. Such an unhealthy dietary style would promote intestinal inflammation by dysregulating the immune system, altering intestinal permeability and the mucosal layer, and resulting in gut microbiota dysbiosis [14]. Compared with traditional medical treatment, changing dietary style brings fewer side effects. Therefore, a plethora of studies have been conducted to investigate the most effective "dietary composition" to treat IBD.

In 2011, a group of Spanish scientists proposed that people who live in western industrialized countries have a higher risk of developing IBD [15]. To test the hypothesis, they conducted a case-control study on 484 volunteers. Two hundred forty-two are IBD patients (with 105 CD and 137 UC) aged >18 and have been diagnosed with IBD in the past 10 years. The other half of the volunteers are healthy controls, matched by sex, age, and smoking habits, and no family history of IBD. All the selected volunteers have at least one year of immigration history in other countries. They found that IBD patients who have emigrated to European countries are more likely to develop IBD than controls who are not resident in western countries [15]. In contrast, the frequency of developing IBD for patients who immigrated to Latin America does not significantly differ from those in the control group. The epidemiology study on IBD has also confirmed that the Western developing countries warrant an increased risk of developing IBD than other parts of the world. Most of the population of the Western diet had higher intake of fat, while South American and Asian people are considered to have healthier eating habits than Westerners. Therefore, several groups of researchers hypothesized that diet might contribute to the etiology of IBD. A prospective 125,445 population-based cohort tested this hypothesis. In this study, 224 volunteers were diagnosed with CD, and 97 have developed UC for more than 14 years. All the participants were asked to answer health-related and dietary questionnaires. A followed-up principal component analysis has identified five dietary patterns that are related to the etiology of IBD: high intake of snacks, prepared meals,

nonalcoholic beverages, and sauces, along with low vegetables and fruit consumption, was associated with a higher likelihood of CD development (odds ratio [OR]: 1.16, 95% confidence interval [CI]: 1.03-1.30, $p = 0.013$). A pattern comprising red meat, poultry, and processed meat was associated with an increased likelihood of UC development (OR: 1.11, 95% CI: 1.01-1.20, $p = 0.023$). A high diet quality score was associated with decreased risk of CD (OR: 0.95, 95% CI: 0.92-0.99, $p = 0.009$) [16]. These findings were confirmed by two case-control dietary studies, one on a high-fat diet and the other one focus on high carbohydrates accompanies with low fiber diet. By studying different fats, scientists found that ω-6 PUFA is proinflammatory while ω-3 PUFA is anti-inflammatory. In Western countries, daily diets always contain unbalanced amounts of ω-6PUFA and ω-3 PUFA. As a result, taking higher amounts of ω-6 PUFA increases the risk of developing IBD in the Western population. Additionally, long-chain triglycerides (LCT) in low-quality food also promote intestinally lymphocyte proliferation and upregulate proinflammatory mediators such as IFN-γ, IL-17, and IL-22 [17].

Due to the onset of proinflammatory signals, the host immune system will start to increase the gut permeability, altering the microbiome inside [18]. Therefore, long-term taking a number of unhealthy fats in their diets is more prone to develop IBD in Western populations than others. High carbohydrates and low fiber are different typical signatures of Western eating habits. A high carbohydrate intake will favor the growth of pathogens and bacteria, resulting in gut dysbiosis. Fibers have many positive effects on the human gut environment. For example, it can promote microbiota diversity, preserve mucosal barriers, and prompt the production of SCFA that, in turn, positively modulate intestinal homeostasis. Thus, lacking fiber in the diet will adversely increase the likelihood of developing IBD [19]. With the increasing understanding of the relationship between diet and IBD, dietary intervention has become a popular strategy in preventing and treating IBD in clinical practice.

2.2. Genetics as a Nonmicrobiome-Related Factor. Genetics is another factor that is considered to associate with IBD incidence. Between 1977 and 2011, Danish scientists kept collecting data from the entire Danish population to establish the IBD patients' family ties. Using statistical analysis, they found that up to 12% of IBD cases are family cases, especially among the young individuals [20]. Since 2012, researchers have identified 230 single nucleotide polymorphisms (SNPs) associated with IBD by conducting GWAS [21, 22]. Among these SNPs, two are the most closely related to the incidence of IBD, showing a significantly high OD value.

The first risk variant for IBD was in the nucleotide oligomerization domain containing the protein two genes (*NOD2*), with the highest OR of 3: 1 in populations with CD. Scientists have identified 200 genetic risk loci and over 30 nonconservative mutations in the NOD2 gene. Many lines of evidence indicate that different types of mutations in the NOD2 gene results in various function lost in the NOD2 protein, increasing the incidence of different types of IBD. For instance, in 2001, Hugot et al. first suggested that the one frameshift and two missenses in *NOD2* gene contribute to the pathogenesis of Crohn's disease (CD) [23]. *NOD2* gene encodes Apaf-1/Ced-4 superfamily of monocytes' apoptosis regulators. They are the product of *NOD2* that activates the NF-κB-inflammation mediating pathway [24]. The activation function of the NOD2 gene product is negatively regulated by its carboxy-terminal leucine-rich repeat domain. NOD2 variants confer CD by altering the structure of the domain or the adjacent domain of the leucine-rich repeat region so that the NOD2 protein can no longer activate the expression of the NF-κB signaling pathway, resulting in lower production of proinflammatory cytokines, leading to reduced bacterial clearance and loss of mucosal barrier function [23, 25]. However, the precise domain that the *NOD2* gene product binds to remains unknown.

Further studies in 2003 identified muramyl dipeptide (MDP) as minimal bioactive peptidoglycan (PGN) motif common to gram-negative and positive bacteria. *NOD2* gene product binds to MDP and activates the NF kB pathway [26]. It has been shown that NOD2, as an innate immune cytoplasmic protein receptor, is expressed by dendritic cells (DCs), phagocytes, and some intestinal epithelial cells. Therefore, when the NF-κB pathway is overexpressed in patients with mutated NOD2 receptors, the innate immune response will be triggered and cause inflammation in the gut [27] (Figure 1).

Lesage et al. found that three SNPs in the NOD2 gene are particularly associated with the incidence of CD. This is accompanied by SNP8 located in exon 4 (c.2104C>T, p.Arg702Trp), SNP12 in exon 8 (c.2722G>C, p.Gly908Arg), and SNP13 in exon 11 (c.3019_3020insC, p.Leu1007fs) [28]. By conducting further investigation, a recent research group found that different haplotypes of risk alleles are associated with the other onset of IBD. For instance, Both UC and CD had an excess of the c.2722G>C and c.3019 3020insC alleles, which are typically associated with a deficiency in peptidoglycan recognition and a failure to trigger the corresponding immune responses [29]. The late-onset form of CD is associated with the T-C-G-C-insC, T-C-G-T-insC, and T-T-G-T-wt haplotypes (OR = 23.01, 5.09, and 17.71, respectively), whereas T-T-G-T-wt and C-C-G-T-wt were only found in CD children (OR = 29.36 and 12.93, respectively; p value =0.001). In conclusion, the mutual allele in all predisposing haplotypes in CD children was the c.2798 +158T, while the presence of c.3019 3020insC and c.802C>T occurred as the most fundamental contributing diplotype in late-onset CD form. Researchers found that 92 probands with biallelic rare and low-frequency NOD2 variants accounted for approximately 8% of our cohort, suggesting that a Mendelian disease inheritance pattern may exist among IBD patients. To confirm this hypothesis, whole genome sequencing was performed in a cohort of 1,183 patients with pediatric-onset IBD (ages 0-18.5 years). They also looked into the role *NOD2* alleles with recessive inheritance play in adult IBD patients from a large clinical population cohort. In this adult IBD cohort, they discovered that

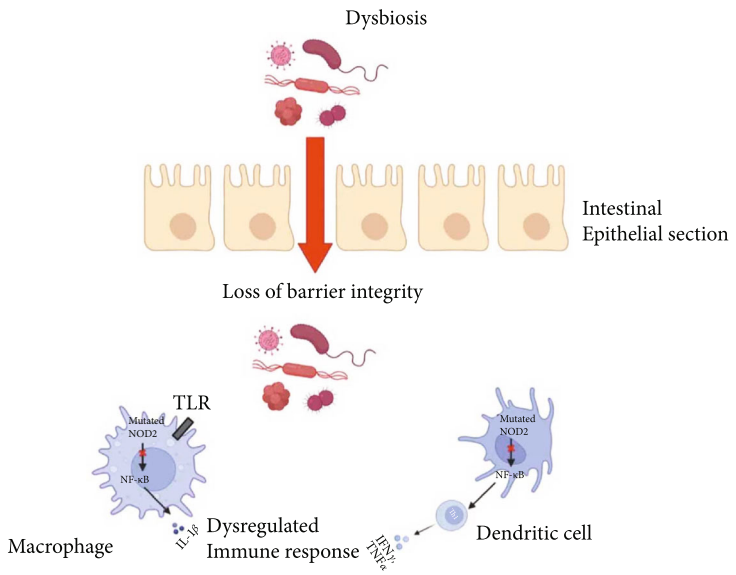

Figure 1: Mutated NOD2 gene product will no longer activate the NF-κB pathway in macrophages and dendritic cells so that no inflammatory cytokines can be released to clear the pathogens, leading to the loss of barrier integrity. As soon as the barrier function was destroyed, more pathogenic microorganisms will enter the intestine, causing dysbiosis, and trigger the onset of IBD.

recessive inheritance of *NOD2* variants accounts for 7% of cases, including 10% of CD cases, confirming the findings from the previous pediatric IBD cohort [30]. Additionally, several patients were diagnosed with IBD before age 18. Therefore, they proposed that the recessive inheritance of NOD2 is a critical mechanical driver of the early onset of IBD. The exact mechanisms of how NOD2 influences the susceptibility of IBD requires additional investigation.

Apart from NOD2, the interleukin 23 receptor (*IL23R*) risk allele also has a high OR of 2.0 in IBD [21]. *IL23R* gene was found in 2006. After a series of statistical filtration, Duerr et al. found that one of the three markers, rs11209026 ($p = 5.05 \times 10^{-9}$, corrected $p = 1.56 \times 10^{-3}$), is a nonsynonymous SNP (c.1142G>A, p.Arg381Gln) in the *IL23R* gene (GenBank accession: NM_144701, GeneID: 149233) on chromosome 1p31 [31]. *IL23R* gene codes for a subunit of the receptor for IL-23 are expressed by the activated T and myeloid cells. Experiments on mice models have shown that IL-23 can stabilize or maintain the Th17 cells, a $CD4^+$ T cell that can release IL-17 cytokine. Increased neutrophil recruitment into tissues and macrophage production of inflammatory cytokines are typical results of IL-17 accumulation, which can further activate stromal, endothelial, and epithelial cells to produce cytokines and chemokines [32]. Thus, in IBD patients, higher levels of IL-17 are an inflammatory signal, triggering the onset of IBD. As a result, Duerr and his team proposed that the IL23R-coded IL-23 receptor on activated T or myeloid cells can affect the IBD incidence via controlling the Th17 cell releasing IBD marker, cytokine IL-17. More importantly, the depletion of IL-23 decreases proinflammatory factors but does not attenuate any systemic T-cell inflammatory responses. This finding supports IL23 as a driver of the innate immune response [32].

Despite extensive evidence linking genetic variations in *NOD2* and *IL23* to IBD, a subset of populations with mutant *NOD2* and *IL23* do not develop IBD. This contradictory phenomenon requires additional investigation. Overall, modifying genetic mutation has become a future therapeutic target for treating IBD.

Apart from *NOD2* and *IL23* genes, the hypoxia-inducible factor (HIFs) gene and its association with IBD are another research focus. Hypoxia-inducible factors (HIFs) are transcription factors activated under hypoxic and pathological conditions. HIFs are composed of two subunits, with an unstable and oxygen sensitive α-subunit (e.g., HIF1α, 2α, and 3α) and a constitutively stable and expressed β-subunits (HIF-1β) [33]. α-subunit can be hydroxylated by prolyl hydroxylases (PHDs) and factors inhibiting hypoxia-inducible factor (FIH) in normoxia (Figure 2(a)). While asparaginyl hydroxylation prevents HIF from interacting with the coactivators cAMP-response element binding (CREB) protein (CBP) and histone acetyltransferase p300, prolyl hydroxylation causes proteasomal degradation of the HIF subunits (p300). The enzymatic activity of PHDs and FIH is inhibited during hypoxia, which stabilizes HIF subunits. Once they have been transported to the nucleus, they form a complex with their subunit, pull in p300 and CBP and then bind to hypoxia-responsive elements (HREs) in the promoters of target genes to activate transcription [33] (Figure 2(b)). One of the typical immunological features of IBD is hypoxia, a decreased oxygen tension at inflammatory sites of GI track. Hypoxia increases the survival of myeloid cells so that those cells can release more proinflammatory cytokines, such as TNFα, which can destroy the tight junction protein between epithelial cells, enhance the apoptosis epithelial cell, and increase the permeability of the gut barrier. Subsequently, epithelial cells under hypoxia conditions

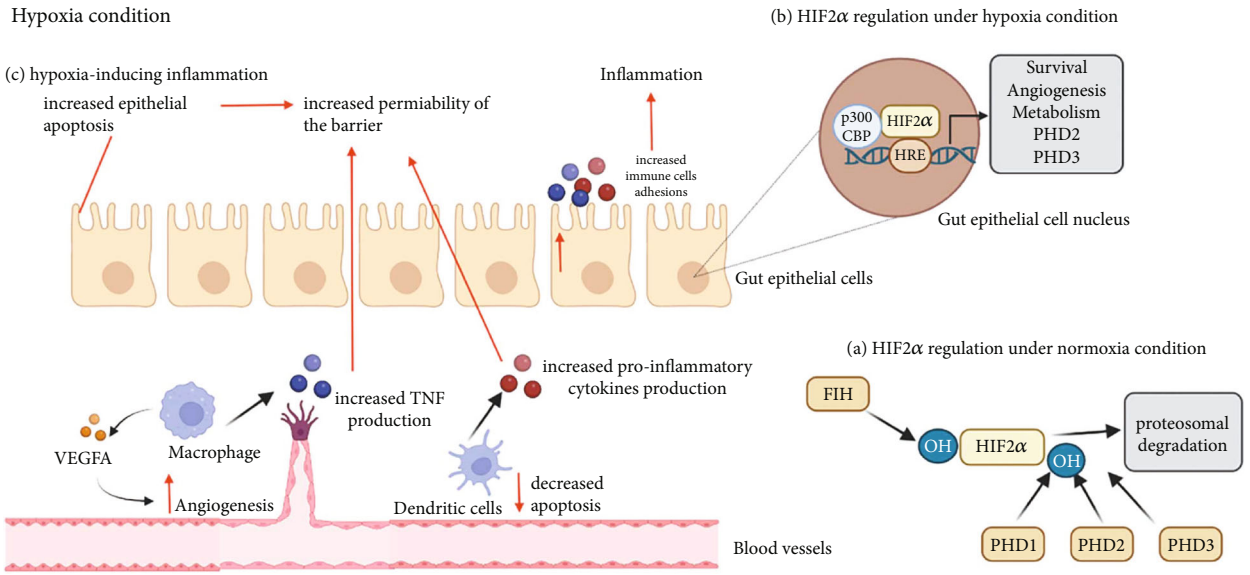

Figure 2: (a) Under the normoxia condition, HIF2α will be hydroxylated by PHDs and FIH, resulting in proteosomal degradation. (b) Under hypoxia condition, enzymatic activity of PHDs and FIH is inhibited during hypoxia, which stabilizes HIF subunits. Once they have been transported to the nucleus, they form a complex with their subunit, pull in p300 and CBP, and then bind to hypoxia-responsive elements (HREs) in the promoters of target genes to start transcription. (c) Hypoxia increases the survival of myeloid cells so that those cells can release more proinflammatory cytokines, such as TNF, which can destroy the tight junction protein of the epithelial layer as well as increase the chance of epithelial cell apoptosis, together increasing the permeability of the gut barrier. Subsequently, epithelial cells under hypoxia conditions are more likely to secrete more cell-adhesion molecules and proinflammatory mediators, attracting more immune cell adhesion and causing inflammation. Additionally, hypoxia condition also can promote the macrophage cells to produce more proangiogenic factor vascular endothelial growth factor A (VEGFA). All the factors attributed to the accumulation of inflammation at the colitis site trigger the onset of IBD.

are more likely to secrete more cell-adhesion molecules and proinflammatory mediators, attracting more immune cell adhesion and causing inflammation.

Hypoxia can also promote the macrophage cells to produce more proangiogenic vascular endothelial growth factor A (VEGFA). All the factors attributed to the accumulation of inflammation at the colitis site trigger the onset of IBD [33] (Figure 2(c)). As we mentioned, HIF1α and HIF2α (also known as Epas1) mediate hypoxia in our bodies. In particular, the role of HIF2α in IBD pathogenesis by inducing inflammation at colitis has attracted the most attention. In 2013, Xue et al. experimented on three groups of DSS-induced colitis transgenic hypoxia reporter mice: mice with conditional overexpression of Epas1 (Epas1 (LSL/LSL)), mice with intestinal epithelium-specific deletion of Epas1 (Epas1 (ΔIE)), or wild-type littermates (controls) [34]. They collected and analyzed the colon tissues from these three groups of mice and IBD patients. In both IBD and colitis-induced mice models, they have observed a significant increase in hypoxia and Epas1. In addition, they noticed that Epas1 (ΔIE) mice had reduced colonic inflammation and were protected against colitis. Intestine-specific overexpression of Epas1, but not HIF1α, increased susceptibility to the induction of colitis by C rodentium or DSS and decreased survival times compared to the control group [34]. The experimental evidence illustrated that HIF2α upregulates the accumulation of inflammation under hypoxia conditions and increases the onsite of IBD (Figure 2(b)). Overall, HIF2α is a crucial target for revealing the relationship between IBD pathogenesis and anti-HIF2α molecules, which have the potential to become another therapeutic target.

2.3. Host Immune System as a Nonmicrobiome-Related Factor. Scientists in the past decades underestimated the impact of immune systems on the pathogenesis of IBD. The disruption of immune homeostasis, especially the imbalance between proinflammatory and anti-inflammatory cytokines, is associated with a higher incidence of IBD, proposed for the first by Powrie et al. in 1994 [35]. Proinflammatory cytokines are the cytokines that control the onset, progression and, ultimately, the resolution of inflammation. Anti-inflammatory cytokines, on the other hand, are often responsible for attenuating inflammation. The proinflammatory cytokines released from CD4+ T cells are characterized with 200-fold more IFNγ and 10-fold higher IL-3 from the mice model of IBD than the healthy controls. The treatment of anti-IFNγ antibodies could attenuate the inflammatory symptoms [35].

The other potential inflammatory cytokine related to the incidence of IBD is tumor necrosis factor (TNF). Anti-TNF antibody was given to IBD mice weekly, and most of the IBD mice maintained stable weights and were protected from sever pathological phenotypes. Even though anti-TNF treatment has shown great success in attenuating IBD symptoms in mice models, its effect is not long-lasting and effective enough; 8 of the mice have developed mild disease [35]. There is another treatment using anti-IFNγ. Compared with anti-TNF treatment, anti-IFNγ is considered to be a more

effective and long-lasting therapeutic approach. None of the mice have developed mild diseases in the later stage. Despite the impacts of TNF as a cytokine on IBD pathogenesis, its receptor (TNF-R) also influences the onset of IBD, especially in CD. By constructing a transgenic mouse overexpression of TNF-R2 and inducing IBD to it, Holtmann et al. observed an early appearance of IBD symptoms and a more severe colitis inflammation in the transgenic mice [36]. They concluded that regulating TNF-R2 signaling is crucial for slowing down the disease exacerbation in Th1-mediated chronic colitis. Moreover, not only are the receptor types of TNF associated with IBD but the solubility of TNF also affects the IBD incidence. There are two types of TNF: membrane TNF (mTNF) and soluble TNF (sTNF). To test the hypothesis, a research group experimented on four groups of immunodeficient mice. They first induced colitis in the mice. Mice were given XENP1595, phosphate-buffered saline (PBS), anti-TNF monoclonal antibody (mAb), or isotype control twice a week after the symptoms had developed. XENP1595 is a dominant negative mutant of TNF, which can neutralize the sTNF. mAb can block both mTNF and sTNF. Research evidence shows that while anti-TNF mAb-treated mice gained weight quickly after the first dose of treatment, mice treated with XENP1595 did not alter the course of the disease. Compared to isotype control-treated animals, mice given anti-TNF mAb had less colon inflammation. Similar levels of colon inflammation were present in XENP1595-treated mice as in PBS-treated ones. The results indicate that neutralization of mTNF is crucial for the treatment of IBD [37]. The experimental finding was confirmed by clinical trials conducted on sTNF receptor etanercept and onercept. In a randomized placebo-controlled trial, at four weeks (response: 39% vs. 45%, $p = 0.763$; remission: 9% vs. 20%, $p = 0.39$) and eight weeks (response: 30% vs. 30%, $p = 1.0$; remission: 13% vs. 25%, $p = 0.44$) of treatment, etanercept was not effective than placebo for either response or remission. A phase II of randomized placebo-controlled dose-finding trial was used to evaluate onercept. Across all dose groups in this study, onercept had no significant impact on clinical remission [38].

rIL-10 has also been suggested to be able to attenuate the symptoms of IBD. By treating the IBD-affected mice daily with rIL-10, Powrie et al. found that 12 out of 19 mice did not develop IBD symptoms in the colon. In other words, rIL-10 has the potential to become another therapeutic target in the treatment of IBD [35]. Therefore, many studies have focused on investigating rIL-10 treatment. Most rIL-10 treatment requires patients to take recombinant human IL-10 (rhuIL-10). Despite the encouraging results in animal experiments, clinical trial results are disappointing. Schreiber et al. conducted trials involving 329 refractory therapy patients with active Crohn's disease, and no significant differences in the induction of clinical remission were seen between placebo and rhuIL-10 at any administrated dose (1, 4, 8, and 20 μg/kg) [39]. More importantly, according to a comparative study, some patients who take rhuIL-10 daily have developed headaches, fever, and anemia as side effects [40]. This line of evidence raises public concerns about whether IL-10-related treatment is efficient and safe. As a result, more investigations need to be focused on improving the clinical trial results and reducing side effects in the future. IFNγ, TNF, and RIL-10 are all mainly released by Th1 cells. Thus, we can conclude that interrupting the cytokine release from the Th1 cells can be a therapeutic strategy for treating IBD. However, this conclusion was incomplete due to different types of IBD responding differently to IFNγ. Scientists proposed that the attenuated function brought by IFNγ is only restricted to CD rather than UC. In treating UC, increasing the secretion of IL-5 is another good option [41].

Similar to Th1, Th17 is another T effector cell that also releases proinflammatory cytokines that influence the severity of IBD. There are five subtypes of Th17-released cytokines. Among all, IL-17A and IL-17F are the two significant cytokines that are associated with IBD. IL-17A primarily plays a protective and anti-inflammatory role in the intestine community by recruiting neutrophils. However, in a research when scientists transferred purified $CD45RB^{hi}$ $CD25^{-}CD4^{+}$ T cells from $Il17a^{-/-}$ mice or wild-type mice into $Rag1^{-/-}$ recipients, they found that $Il17a^{-/-}$ T cells receive cohorts had developed aggressive inflammatory disease compared to that of recipients of wild-type cells, as shown by their accelerated decrease in body mass [42]. However, abnormal Th17 cell proliferation would induce myofibroblasts to secrete matrix metalloproteinases (MMPs), a family of proteases that penetrate various parts of the extracellular matrix and cause damage to epithelial cells, resulting in gut inflammation and an increased incidence of IBD [43]. This was supported by detecting the large amount of Th17 in the peripheral blood of IBD patients [43]. The overexpression of IL-17A can also bind with TNF-α to exacerbate the inflammatory response of IBD. Th17 also secretes IL-21, which forms a positive feedback loop with IL-17, improving the production of IL-17 and increasing the severity of IBD [44]. Genetic evidence also shows that several IBD-related genes such as IL-23R, CCR6, and Act1 also participate in regulating IL-17 signal transduction pathway. These data strongly implies that IL-17 secreted by Th17 is a bifunctional regulatory cytokine that may help understand the pathogenesis of IBD. More research is needed due to the complicated immunological pathways mediated by Th17.

Antigen-presenting cells include macrophages and lamina propria dendritic cells (DCs) and also release proinflammatory cytokines, such as IL-1β, IL-6, IL-18, and TNF, which aggregate the IBD syndrome. As for IL-1 cytokine families, keeping a balanced ratio between IL-1 and IL-1 receptor antagonists (IL-1ra) is the key to preventing the chronicity of IBD [45]. This was proved by measuring the ratio of IL-1 and IL-1 receptor antagonists (IL-1ra) in IBD patients and controls, which revealed that the balance in the former group is significantly lower than the healthy controls. In terms of IL-6, which binds to soluble IL-6R (sIL-6R) and inhibits apoptosis of mucosal T cells, the increasing resistance of T cells, especially T helper one cells, would alleviate the release of proinflammatory such as TNF and IFNγ to perpetuate the IBD [46].

The effect of cytokines produced by innate lymphoid cells (ILCs) on IBD has also been reported. ILCs control

innate mucosal immunity by releasing proinflammatory cytokines such as IFNγ, IL22, and IL-17, which are the vital proinflammatory cytokines described above [17]. The activation of the ILCs is mediated by IL-23, which has been revealed in mouse models. A study published in 2011 proposed that ILCs may serve as a novel tissue-specific target for IBD subtypes and contribute to intestinal inflammation through cytokine production, lymphocyte recruitment, and organization of the inflammatory tissue. To test the hypothesis, they conducted a clinical trial: comparing the expression of ILC-related cytokines between IBD patients and healthy individuals. Their results confirmed that intestine of CD patients' contains more IL-17-producing ILCs than healthy controls. However, similar results are not seen in UC patients [47]. The contradicting amount of ILCs present in two types of IBD emphasizes the importance of specializing treatments in clinical trials.

So far, the major proinflammatory cytokines and their impacts on the pathogenesis of IBD have been described. In contrast to proinflammatory cytokines, anti-inflammatory cytokines such as IL-10 and transforming growth factor-β (TGFβ) exert protective roles in curing IBD. Regulatory T cells (Treg) are a significant source of anti-inflammatory cytokines. This was proved by knocking out the *Treg*-coded gene in mice. *Treg*-deficient mice fail to produce either IL-10 or TGFβ to suppress the activation of proinflammatory cytokines produced by effector T cells or ILCs. As a result, those knockout mice soon developed IBD [48]. Therefore, increasing the number of anti-inflammatory cytokines to balance the proinflammatory cytokines has now been the central therapeutic insight for treating IBD. However, some IBD patients are resistant to anti-inflammatory cytokines, especially TGFβ. In further investigation, scientists found that TGFβ-resistance patients' effector T cells highly express SMAD7, which is responsible for inhibiting TGFβ signaling.

As for TGFβ resistance patients, a new therapeutic strategy is oral intaking SMAD7 antisense oligonucleotides [49]. Mongerson is one of the most well-known oral SMAD7 antisense drugs. Its efficiency in treating CD was examined in a double-blind, placebo-controlled, phase II trial, randomly assigning patients to receive 10, 40, or 160 mg of mongers or placebo per day for two weeks. This study found that participants with Crohn's disease who received mongers had significantly higher rates of remission and clinical response than those who received a placebo [50]. However, the result from a multicenter, randomized, double-blind, placebo-controlled phase III was not as impressive as phase II. In phase III trials, at week 12, the primary endpoint-clinical remission was achieved by 22.8% of GED-0301 patients versus 25% of placebo patients ($p = 0.6210$). When the study ended, the percentages of patients who had reached clinical remission at week 52 were similar between each GED-0301 group and the placebo [51]. As a result, further clinical trials are expected to conduct and confirm the efficiency of these drugs.

Different cytokines have different functions at various IBD stages. For example, as for the IL-1 cytokine family, increasing granulocyte recruitment and ILC activation, IL-1 promoted innate immune pathology in intestinal inflammation brought on by Helicobacter hepaticus. In addition, IL-1R signaling in T cells controlled the early accumulation and survival of pathogenic CD4+ T cells in the colon in the T cell transfer model of colitis [52].

As the binding site of IBD is in the intestine and colon, the interaction between the gut microbiome and immune effects is not mutually exclusive. The human intestine is covered by intestine epithelial cells (IECs), which enhance intestine barrier function and heal inflamed mucosa for IBD patients via cytokines [53]. By activating specific signaling pathways in IECs, gut microbiota and its byproducts can impair the integrity of the gut barrier. In contrast, IECs can act as a barrier between the host immune system and the gut microbiota to prevent an overactive immune response and control the gut microbiota's composition by offering an alternative energy source and releasing some molecules, such as hormones and mucus [54]. Not just IECs but also intestinal dendritic cells also closely interact with the gut microbiome. A previous study proposed that the composition of the intestine microbiome influences the expression of the cytokines released by intestinal dendritic cells (DCs).

To test the hypothesis, Ng et al. analyzed the expression of cytokines, including TLR-2, TLR-4, (IL)-10, IL-12p40, and IL-6 from isolated DCs of 28 IBD patients and ten controls via flow cytometry. Then, they also analyzed the intestine microbiota from fecal samples using 16 s rRNA. The ratio of *Bacteroides*:*Bifidobacteria* was correlated with IL-12p40(+) DC ($r = 0.535$, $p = 0.003$). *Bifidobacteria* and IL-10(+) DC correlated, while *F. prausnitzii* and IL-6(+) DC did not ($r = -0.50$; $p = 0.008$). TLR-4 levels on DC had a poor correlation with *F. prausnitzii* concentration. 43 establishing novel strategies for preventing and treating IBD may be made possible by understanding the interactions between the gut microbiome and the host immune system. The cytokines, immune cells that play a major role in IBD pathogenesis, are summarized in Table 1.

2.4. Other External Factors. Except for the three primary factors mentioned above, other factors, such as smoking, age, gender, and ethnicity, are closely associated with the development and incidence of IBD. These factors draw less attention because their ability to increase the incidence of IBD does not gain enough support from current evidence, and many exception cases exist. Therefore, we only briefly introduce some statistical data that illustrate their influence on the susceptibility of IBD. As for smoking, British scientists Harries et al. first found that nonsmokers are less likely to develop UC than healthy controls [60]. Another study confirmed the connection between smoking and the incidence of CD and proved that the incidence of CD is affected by the frequency of smoking. After a meta-analysis concluded that current smokers are less likely to develop CD than former smokers with risk factors 3.5 and 4.8, respectively, compared with healthy controls, whether present or former smokers, they both have a higher risk of developing CD [61]. According to related data, in Western countries, 20-30 is the peak age interval for diagnosing IBD. Another peak happens during ages 60-79 [62]. As for the Asian population, the onset of IBD seems to be earlier than Westerners, with the peak at 20-24 and 40-44 years, respectively [63].

Table 1: This table summarized the major IBD-related cytokines, showing their cellular source and explaining their IBD pathogenesis role.

Major cellular sources	Roles in IBD	References
CD4+ T cell, ILCs	(i) Increases permeability of intestinal vessels by disruption of VE-cadherin junctions, associated with increased inflammation and progression of IBD (ii) IFNγ deficient mice show attenuated IBD symptoms	(i) Langer et al., [55] (ii) Powrie et al. [35]
Th1 cell	(i) Increased TNF-alpha levels have been demonstrated in studies of patients with ulcerative colitis (ii) Anti-TNF-alpha therapy is effective in ulcerative colitis (iii) TNF binds to TNF receptor will activate c-Jun and NF-κB transcription factor. These two transcription factors involve in many inflammation encoded gene. As a result, induce inflammation at colon sites	(i) Pagnini and Cominelli, [56] (ii) Schmitt, Neurath and Atreya, [57]
Th1 cell, intestine epithelial cells, Treg	(i) Inhibits both antigen presentation and subsequent proinflammatory cytokine release, resulting an unbalance between proinflammation and anti-inflammation cytokines	(i) Li and He, [40]
Th17 cell, ILCs	(i) IL17 signaling is able to induce a cascade of proinflammatory molecules like TNF, IFNγ, IL22, lymphotoxin, IL1β, and lipopolysaccharide (LPS). IL17A is known to mediate signaling synergistically to drive expression of inflammatory genes (ii) Activated by IL-23 pathway as followed: when IL23 binds to its receptor, Jak2 and Tyk2 kinases are activated. This phosphorylates the receptor to create a docking site, which then causes STAT3 for the p19 subunit and STAT4 for the p40 subunit to be phosphorylated. The transcription of several effector cytokine genes in CD, including IL17A, is triggered by the activation of several pathways by the IL23R receptor	(i) Schmitt, Neurath and Atreya, [57]
Th17 cell	(i) IL-21 enhances NK cell activation and induces Th17 cell differentiation in IBD (ii) The increased expression of IL21 gene was seen in UC patients	(i) Solaymani-Mohammadi et al., [58]
Regulatory T cells (Treg)	(i) Active TGF-β binds to its receptor and regulates mucosal immune reactions through the TGF-β signaling pathway. Dysregulated TGF-β signaling is observed in the intestines of IBD patients	(i) Ihara, Hirata and Koike, [59]

Due to the differences in the onset age between east and west population, several research groups suggested that incidence of IBD might also be related with the race [2]. According to data provided by the Rochester Epidemiologic Project, White people had an annual incidence rate of IBD of 21.6 cases per 100,000 person-years (95% confidence interval (CI), 20.0-23.1), while non-White people had an annual incidence rate of 13 patients (95% CI, 8.3-17.5). The significant difference between White and non-Whites shows that White is more susceptible of IBD than non-White [2]. This might be due to their diet habit, as mentioned above.

3. Microbiome-Related Factors

The first construction of the gut microbiome could be a throwback to the infantile period. The specific compound in breast milk is suggested to be the main driven force for constructing the infantile gut microbiome. At that time, our gut microbiota population was dominated by *bifidobacterial spp*., rapidly expanding their population size by breaking down the specific glycan in human milk [64]. In the later stage of life, the gut microbiota is dominated by *Bacteroidetes* and *Firmicutes*. During different developmental stages of life, many external factors can individually influence the composition of the gut microbiome, resulting in a differentiation of microbiome composition.

The external factors include mode of delivery, gestational age, breastfeeding or formula milk, maternal diet, and host genetic factors [64]. During the development of the number of healthy microbiota such as *Bifidobacterium* species, Escherichia coli, *Faecalibacterium prausnitzii* and lactobacillus species is greater than the pathogenic bacteria such as Enterococcus faecalis, *Methanobrevibacter smithii*, *Clostridium difficile*, and *Campylobacter*, and the individual will be placed at a low-risk position for developing gastrointestinal diseases. However, suppose the abundance of harmful bacteria overtakes the healthy bacteria. In that case, the individual will be more likely to be suffered from intestinal and metabolic disorders, such as IBDs, diabetes, and obesity. A healthy adult gut microbiome comprises mainly three forms of life: fungi, viruses, and bacteria. Bacteria account for 99% of the gut microbiome species and are followed by fungi (0.1%). The virus only occupies a tiny amount of the whole microbiome (0.001%) [65]. These three living forms interact during the IBD pathogenesis. Among them, bacteria directly influence IBD pathogenesis. The other two forms indirectly affect the disease pathogenesis by affecting the composition of the gut bacteria community. The detailed mechanisms of how they interact and affect each other, finally contributing to IBD, have been well established and studied in the past decades. In this review, we will further classify gut microbiota into three main categories: bacteria, fungi, and viruses, discussing in detail how they mediate

the pathogenesis of IBD. We will also discuss the interaction between gut microbiota and other external factors, especially the host immune system and genes.

3.1. Bacteria. Bacteria species occupy almost 99% of the gut microbiota population. Researchers have identified three main categories of bacteria in the human gut by comparing the fecal microbiota sample between IBD patients and healthy controls. One is probiotics such as *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia*, which can help with digestion and maintaining gut homeostasis [66]. The second type is opportunistic bacteria which only infect when the host is weak/unhealthy. These include *Escherichia coli* and *streptococcus* [67]. The third type is inflammation-inducing bacteria which will increase the risk of IBD, such as *Proteobacteria*, *Bifidobacterium sp.*, adherent/invasive, group IV and XIVA *Clostridium*, *Fusobacterium* species, *Faecalibacterium prausnitzii*, *Ruminococcus gnavus*, and *Roseburia* species. According to clinical and experimental data, IBD patients are always accompanied by intestine lesions in the area with the highest bacteria diversity (the colon) [10]. More importantly, by sequencing and comparing the bacteria composition of IBD patients and healthy controls, the infected site of IBD patients' guts is predominated by opportunistic and inflammation-inducing bacteria rather than beneficial probiotics [68]. Therefore, it has been proposed that the dysbiosis of the gut microbiota is responsible for the leading cause of IBD. Many studies have been conducted to test the hypothesis, and the results are convincing, indicating a relationship between microbiota dysbiosis and IBD. For example, Ohkusa et al. have isolated *Fusobacterium varium* from UC patients' fecal samples. When they introduced the isolated *Fusobacterium varium* strain to mice, mice showed human ulcerative colitis-like lesions after 24 hours [69]. Gevers et al. analyzed and compared the fecal samples collected from CD patients and healthy controls and found that bacteria strains, including *Pasturellaceae*, *Veillonellaceae*, *Neisseriaceae*, *Fusobacteriaceae*, and *E. coli*, have shown an increased tread. On the other hand, the abundance of strains such as *Bacteroides*, *Clostridiales*, *Faecalibacterium*, *Roseburia*, *Blautia*, *Ruminococcus*, and *Lachnospiraceae* has decreased [70]. Additionally, from the genetic level, scientists found that some genes related to the interaction between the gut microbiome and the host environment are mutated, such as the NOD2 gene, which has been discussed in the gene section above. Many other similar experimental data from animals and humans support the hypothesis, which will not be discussed in detail. In general, all of the support results tend to suggest a relationship between microbiome and IBD. However, there is no blinded, controlled investigation that confirms a specific microbiota is linked to IBD. As a result, we cannot identify the causal relationship between the gut microbiome and IBD: whether the change of microbiota observed in IBD patients is due to the inflammation triggered by IBD or the change of microbiome leads to/accelerates the onset of IBD [71].

The dysbiosis of the gut microbiome does not affect the pathogenesis of IBD on its own. Instead, it interplays with the host immune system, host gene, and the digestive molecules present in the surroundings together, forming a complex interaction net, triggering the onset of IBD. As for the host immune system, research evidence showed that many bacterial species selectively altered in the dysbiosis accompanied by active IBD. This is well documented for immune functions mediated by *Faecalibacterium prausnitzii*, the subset of *Clostridium* species. *Faecalibacterium prausnitzii* has an anti-inflammatory effect shown both in vivo and in vitro [72]. In vivo, by attaching the NF-κB reporter gene to the Caco-2 cells, Sokol et al. found that *F. prausnitzii* supernatant inhibited the IL-1β-induced NF-κB expression (a proinflammatory-induced pathway). In UV-killed bacteria (control), the NF-κB pathway was normally expressed [73]. This was explained by the butyrate produced by *F. prausnitzii*. Butyrate is an anti-inflammatory short-chain fatty acid (SCFA) that is reduced in IBD patients' mucosa and feces. This was proved by mixing isolated lamina propria cells (LPMC) and peripheral blood mononuclear cells (PBMC) with butyrate and comparing their secretion of tumor necrosis factor (TNF) and mRNA levels with control group cells (PBMC and LPMC only).

Butyrate can effectively decrease TNF production and the expression of proinflammatory mRNA by inhibiting the NF-κB expression via signaling transductor G proteins [74]. Apart from its anti-inflammatory effect, butyrate can also initiate signaling pathway activation or repression by GPCRs, activating HIF-1α, STAT3, and SP1 or repressing (e.g., NF-κB) transcription factors (TFs), increasing epithelial barrier function, antimicrobial peptide (AMPs) production, and cell proliferation, and decreasing inflammation [75]. *In vitro*, peripheral blood mononuclear cell secretes less IL-12 and IFN-γ and more IL-10 after stimulated by *F. prausnitzii*. Additionally, oral administration of *F. prausnitzii* or its supernatant vastly reduced the severity in the TNBS colitis model [73]. Altogether, *F. prausnitzii*, as a probiotic marker bacterial strain, not only interplays with the host's immune function, adjusting the balance between proinflammatory and anti-inflammatory production, but is also associated with the host eating diet: diet riched in butyrate or other types of SCFA can effectively help to strengthen the intestine barrier, against microorganism's invasion, preventing from the onset of IBD (Figure 3).

Other health-benefit gut bacteria (e.g., *Blautia faecis*, *Roseburia inulinivorans*, *Ruminococcus torques*, and *Clostridium lavalense*) have a similar effect on the host immune system by affecting the function of and innate immune cells and mucosal T cells. These strains activate the epigenetic DNA methylation adapter UHR1, which impacts the differentiation and proliferation of Tregs and induces Tregs through IL10, an inducible T-cell costimulator (ICOS) and butyrate. For Treg-mediated immunologic tolerance to the intestinal microbiota to remain in place, these effects necessitate a complete T-cell receptor repertoire. Intestinal microbiota-driven IL17 production and spontaneous colitis occurred in mice with constrained T-cell receptor repertoires, most likely due to impaired regulatory T-cell function. However, a study pointed out that different gastrointestinal disease recipients respond differently to the same probiotic

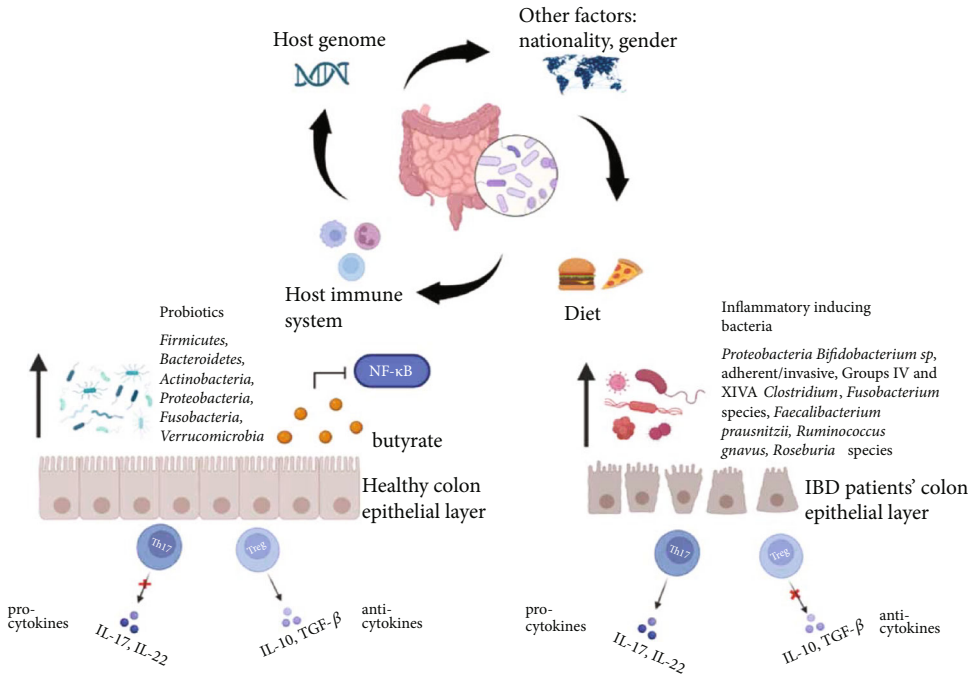

FIGURE 3: The dysbiosis of the gut microbiome does not affect the pathogenesis of IBD. Instead, it interplays with the host immune system, host gene, and the digestive molecules present in the surroundings, forming a complex interaction net and triggering the onset of IBD. Healthy controls' colon epithelial cells contain more probiotics which can effectively be fermented to produce the by-product butyrate. Butyrate can inhibit the expression of the NF-kB pathway, which inhibits the production of proinflammatory cytokines such as IL-17 and Il-22. Probiotics also promote the host immune system to release more anti-inflammatory cytokines. On the other hand, IBD patients' colon contains more pathogenic bacteria species, which will increase the release of proinflammatory cytokines and trigger the onset of IBD.

strain [9]. In other words, the individual host microbiome and the immune system might interact with the probiotic strain, leading to various pathogenesis pathways. Therefore, we could hypothesize that the IBD pathogenesis is related to the common metabolites released by the probiotic strain instead of the probiotic strain.

3.2. Fungi. Fungi only account for around 0.1% of the gut microbiota population by sequencing 3.3 million nonredundant microbial genes via Illumina-based metagenomic sequencing [76]. Fungi locates on almost every mucosal surface of humans, from the oral cavity to the gastrointestinal tract, and their composition varies with the location. Candida genus, which contains about 160 species, dominates the gut fungi community. Regarding species level, in humans, *C albicans*, *C blabrata*, and *C parapsilosis* are the three significant species colonized in the gut. However, for mice, *Candida tropicalis* is dominant [77]. The stability of the gut fungi population is controlled by the surrounding factors, particularly the gut immune system. More importantly, by interacting with and altering the host immune system, the gut fungi population can indirectly influence the pathogenesis of IBD [78]. This was well documented by many studies. For example, Ott et al. found a higher mean fungal diversity in colonic biopsy tissue samples from CD patients compared to healthy controls using metagenomic 18S ribosomal DNA-based denaturing gradient gel electrophoresis [79]. Another study shows similar results, finding a similar fungal diversity in CD patients and healthy controls [80]. However, there are also some contradicting results, showing a decrease of fungal diversity in CD or UC patients [81]. Therefore, whether a reduction or an increase in fungal diversity would impact the incidence of IBD require further investigation. Despite the uncertainties, the mechanism of how fungi influence IBD pathogenesis is straightforward and can be concluded in two main approaches. Gut fungi are described as opportunity invaders, and they are masked by their cell wall ligands, such as chitin, β-glucans, or mannans, when they start evading the host. Once recognized, the host's innate and adaptive systems release cytokines, including IL17 and IL22. IL17 is a typical proinflammatory cytokine, and its overexpression of them would worsen the IBD symptoms. Once the amount of released IL-17 is above the threshold and breaks the balance with the anti-inflammatory cytokines, the host will be exposed to a higher risk of developing IBD [82].

The other way the fungi community affects the IBD is by forming a competitive relationship with the surrounding bacteria population. Fungi are eukaryotes and might have developed metabolic pathways that prokaryotes not employed [83]. This metabolic selective advantage might allow fungi to outcompete some beneficial bacteria species, destroying the constant network between host bacteria and immune system, leading to inflammation response, followed

by increased risk of IBD [4]. Currently, due to the technology limitation, we cannot detect many bacteria at the species level. We cannot distinguish between sexual and nonsexual. Thus, continuous study needs to be taken to gain a complete understanding of the concept of fungi and IBD.

3.3. Virus. Among all types of viruses, bacteriophages are closely associated with the pathogenesis of IBD. Bacteriophages normally invade bacteria or archaea. With time, phage and bacteria have developed a predator-prey relationship. Phages invade bacteria by inserting virion-coded genes into the bacteria's host genome, and bacteria, in response, have developed a CRISPR system to cleave the invading virus gene [84]. There is no evidence directly proving viruses influence IBD pathogenesis. However, in clinical therapeutic experiments, scientists found that prokaryotic virome can gain help from the intestine's immune system, promoting the release of antibacterial cytokines to kill the bacteria population. Those antibacterial cytokines are also proinflammatory cytokines. By doing so, the virus not only encourages the fast growth of itself but also introduces inflammation at the intestine's surface, which might further develop to become IBD [85]. However, as viruses are considered to be absolute parasites, extracting and sequencing virus genomes from fecal samples always fail to count all the virus species. As a result, more studies are needed to develop advanced sequencing method for examining the viral population. Only by doing so can we gain a different understanding of how the virus impacts IBD.

3.4. Microbiome-Based Therapy. Due to the insufficient knowledge and understanding, we have not been able to develop a medicine that can treat patients with IBD completely, particularly for CD. The traditional treatment comprised both medical and surgical treatments. Patients usually start with medical treatments, which focus on controlling the intestinal inflammation caused by the dysbiosis of the cytokines, using an anti-inflammatory drug such as colazal, mesalamine (Asacol, Apriso, Lialda, and Pentasa), olsalazine (Dipentum), and sulfasalazine (Azulfidine). If the symptoms are not controlled properly, they will be passed to biotherapy, taking antibiotics, including infliximab (Remicade), infliximab-abda (Renflexis), and infliximab-dyyb (Inflectra) [1]. This anti-inflammatory drug acts as the inhibitor of the proinflammatory cytokines. For example, infliximab is a monoclonal antibody which acts as an inhibitor of proinflammatory cytokine TNF-α effectively attenuating its release [86]. Many studies have shown that during the anti-inflammatory treatment, both the gut microbiota and its metabolic product compositions are significantly changed [87]. As for infliximab treatment, in a systemic review of 10 studies, Estevinho et al. found the gut microbiome α-diversity of the patients who responded to infliximab have be improved with the increased abundances in genus, including *Faecalibacterium*, *Roseburia*, or *Clostridium* [88]. This is confirmed by another cohort, including Chinese and Western samples. In this study, they found that despite the nationality, patients from both populations show an increase in *Clostridiales* after receiving Infliximab treatment. Apart from *Clostridiales*, the number of bacteria species such as *Bifidobacterium*, *Clostridium colinum*, *Eubacterium rectale*, and *Vibrio* also showed an increasing trend after infliximab treatment [87]. More importantly, the increased abundance of *Clostridiales* can serve as a biomarker for predicting the effectiveness of infliximab treatment with an accuracy of up to 85% [89] During the mild or even severe stages of the disease, patients are recommended to take surgery to remove the infected colon because of the increased risk of colon cancer. For UC patients, surgery can promise a complete cure, but not for CD patients. Traditional treatments have several adverse effects. First, regular antibody use would boost the emergence of antibiotic-resistant microorganisms. Usually, the medical treatment is lifelong, bringing many inconveniences to the IBD patients' daily life. Although surgery can successfully relieve UC symptoms, the stringent selection procedure excludes elderly patients and patients with other pathogenic disorders. Therefore, scientists are always seeking additional treatment that can cure IBD completely. In recent years, increasing evidence supported the microbiome-related treatment to become one of the most awarded future IBD treatments. This section will summarize the current treatments into two categories: correcting dysbiosis and controlling inflammation.

3.4.1. Approach to Correcting Dysbiosis. As we have mentioned, gut bacteria in IBD patients are dominated by inflammation induce bacteria species rather than probiotics. Thus, correcting dysbiosis has been suggested, and to achieve this, three main treatment strategies have been investigated, including phage therapy, probiotic treatments, and fecal microbiota transplantation (FMT). As for phage therapy, scientists genetically engineer a phage that can target the pathobionts in IBD patients' guts and kill them to restore a healthy gut microbiome [90]. A group of scientists first designed a "phage cocktail" containing five different phages that can target the Klebsiella pneumoniae (*Kp*) strain in IBD patients. The "phage cocktail" targets to the all the Kp strains, including the mutated ones, via distinct mechanisms. Compared to the therapy with single phage, the "phage cocktail" can essentially prevent the appearance of phage-resistant bacteria. In animal experiments, all IBD mice administrated with "phage cocktail" attenuated the disease symptoms, proving phage therapy's effectiveness.

Additionally, in a recent phase 1 clinical trial, none of the healthy volunteers showed side effects after taking the "phage cocktail," demonstrating the viability of combination phage therapy given orally in preventing resistance while effectively inhibiting pathobionts caused noncommunicable diseases [91]. This is a new starting point for phage therapy by translating the theories into practice. Further studies are required to be put in investigating more phages, which can target more pathobionts other than Kp, as well as maintaining and improving safety by taking more clinical trials.

Fecal microbiota transplantation (FMT) is another strategy that has therapeutic potential in treating IBD. This is achieved by transporting the gut microbiota from a healthy donor to an IBD patient via fecal transplantation. The

effectiveness of FMT treatment is equivocal due to the limited experimental evidence and low success rate in clinical trials, especially for CD [4]. A systemic review and meta-analysis, which includes 18 studies, in total 122 IBD patients, has been performed to assess the efficiency of FMT [92]. According to the subgroup analysis, CD patients were more likely to respond to FMT than UC patients, with an estimated response rate of nearly 61% of patients achieving clinical remission. This is in contrast to a much lower response rate of 22% in UC patients [93]. A recent study showed a conflicting result which demonstrated that FMT could effectively alleviate remission in UC patients. Their study used two donors: donor one collected over 44, and the second donor (donor 2) was organized on week 70. Interestingly, they found that the efficiency of using donor $1'$s (100% efficiency) stool is significantly higher than using donor $2'$ (35% efficiency). By analyzing microbiota richness and evenness using ß and ∂ diversity tests in these two donor samples, they found differences in the relative abundance of 90 bacterial species and one archaeon, 44 of which were more significant than 0.1% [94]. The shift in microbiota composition over time suggests that metrics other than specific microbial species or metabolites were newly associated with therapeutic efficacy in UC, such as donor microbiota stability and species evenness. Another randomized, controlled trial by Moayyedi and associates confirmed that the effectiveness of FMT treatment for UC patients is comparable to that for CD patients. Their study included 75 UC patients and randomized them to weekly FMT or water enemas for six weeks. On week 7, by evaluating the patients' remission, Moayyedi et al. found that patients who received FMT were significantly more likely to achieve remission than those who received a placebo (25% vs. 5%; $p = 0.03$) [95]. The conflicting results from various studies revealed that efficiency of FMT treatment is not stable enough to be used in the clinic. Fecal microbial stability is influenced by many external factors, such as diet. Therefore, selecting appropriate donors in the future should be placed first in FMT treatments. More clinical results need to be conducted to make FMT a reliable treatment.

Compared to FMT, symbiotic treatments have gained more support in clinical trials. Symbiotic treatments comprised two parts: prebiotics and probiotics. When used separately, probiotics and prebiotics both effectively treat IBD. The effectiveness of probiotic therapy has been well studied for many years. A well-researched probiotic called E. coli Nissle 1917 is as effective as mesalazine at keeping ulcerative colitis in remission. Other specific probiotics with proven effectiveness in IBD include the *bifidobacteria* strains, the yeast *Saccharomyces boulardii*, and *Lactobacillus* GG. VSL#3, and one of the most promising probiotic supplements combines eight bacterial strains (*Lactobacillus casei*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, and *Streptococcus salivarius subsp*). Additionally, after total proctocolectomy and J-pouch development, VSL#3 can prevent pouchitis [96]. A recent study demonstrated that probiotic consortia comprising Lactobacillus Reuters, *Lactobacillus gasseri*, *Lactobacillus acidophilus* (*Lactobacillus* spp.), and *Bifidobacterium lactis* (*Bifidobacterium spp.*) could attenuate the IBD symptoms in the DSS-induced colitis mice model [97] F. *prausnitzii* is shown to have a protective effect on the intestine by producing barrier-enhancing and immunosuppressive SCFAs, stimulating Tregs to produce IL-10 thereby inhibiting exaggerated immune responses in IBD. In multiple mouse models, *F. prausnitzii*, *Clostridia* strains, and *B. fragilis* could reduce the severity of colitis. However, as we have mentioned above, different people respond differently to the same music of probiotics. Thus, personalized probiotics could be a potential investigating field in the future.

Prebiotics are the undigested oligonucleotide in the food, and it is usually used by intestinal probiotics to promote gut microbiota growth and metabolic reactions, fermenting into short-chain fatty acid (SCFA) [98]. Its effect on IBD is less studied compared to probiotics. Prebiotics contribute to the cure of IBD mainly in three approaches. First, research has shown that prebiotics can change the composition of the intestinal microbial community by promoting the development of commensal protective bacteria and improving resistance to colonization with bacteria that cause disease, thereby reducing the severity of colitis. The selective growth is due to two reasons. Only certain beneficial bacteria species have the enzymes to process the fermentation of the prebiotics in the colon. The best study example is *Bifidobacterium infantis*. Therefore, with the selective gaining advantages, probiotics can quickly overtake the pathogens' positions in the colon. The other reason referred that the fermentation conducted by probiotics usually results in a lower pH, which can effectively inhibit the growth of certain pathobionts [*100]*. The SCFA produced by the probiotic fermentation can also lower the colonic pH, inhibiting pathogenic bacteria growth. Additionally, the prebiotics can prevent the harmful bacteria from adhering to and colonize the gut epithelium. The biochemical study revealed that the terminal sugar of prebiotic oligonucleotides could interfere with the pathobionts' receptor, preventing the attachment to the colon epithelial surface. Finally, prebiotics can also balance the production of proinflammatory and anti-inflammatory cytokines, reducing inflammation at the colon site.

There are only two prebiotics that are permitted to be used: one is inulin and the other is FOS [98]. Despite the advantages brought by prebiotics, dose-dependent side effects also exist. Common side effects include abdominal pain, flatulence, bloating, and diarrhoea [100]. The data collected for prebiotic treatments are mainly from animal models. The limited human trials all showed an effective reduction of IBD symptoms. Combining prebiotics and probiotics, symbiotic treatment has gained big success recently. According to experimental data, prebiotics can indirectly stimulate endogenous protective intestinal bacteria while directly increasing the number of protective bacteria in the gut. Before including prebiotics in our standard medical arsenal for IBD, more randomized and placebo-controlled large clinical trials are required [98].

The metabolites of the probiotics can also treat IBD, which is described as a postbiotic treatment. In a recent study, scientists found that by administrating the probiotic

fermented metabolites to the DSS-induced colitis mice model, the symptoms of IBD, such as losing weight and having blood feces, are effectively controlled by the metabolites. However, their effect on bacterial composition and diversity is restricted to the small intestine, indicating that postbiotics are less effective in treating IBD than probiotics [97].

3.4.2. Approach to Controlling the Gut Inflammation. Controlling inflammation is the mainstream strategy used in treating IBD. By targeting the proinflammatory cytokines mentioned in the previous section, IBD patients show a good clinical recovery. Among all the proinflammatory cytokines, TNF-α is the central target. The first anti-TNF drug invented is infliximab (IFX). This chimeric monoclonal IgG1 antibody binds and neutralizes the TNF-α. In the clinic, patients given IFX all perform a certain degree of remission to the IBD [65]. However, the high price of IFX limits its uses in underdeveloped and some developing countries. There are a particular group of patients not responding to the IFX. In this case, they are given an alternative drug named adalimumab (ADA). Proinflammatory cells undergo apoptosis when the ADA specifically binds to TNF molecules, blocking the interaction of this cytokine with its surface receptors p55 and p75. It is beneficial for treating mild and severe CD. Compared to IFX, ADA is considered to be safer [101]. On the other hand, the side effects such as renal complications and delayed hypersensitivity brought by IFX and ADA have become one of the primary concerns, which stops some patients from choosing them. Thus, in the future, reducing the side effects and lowing the cost could be the two major technological boundaries for extending the biomedical practice.

4. Discussion

In this review, we have demonstrated that host gut microbiota interplays with the host genome and immune system together impact the pathogenesis of IBD. Many research groups found that the abundance between probiotics and pathogenic bacteria is unbalanced in IBD patients' guts. Therefore, correcting dysbiosis has become a mainstream strategy for curing IBD. Even though the results were fascinating in the mouse and some human models, some uncertainties still need further investigation to improve the macrobiotic treatment's effectiveness and specificities. Firstly, no experimental evidence exists to prove that specific bacteria strains will lead to higher IBD incidence than others. As a result, we cannot confirm the causal relationship between dysbiosis of the microbiome and IBD pathogenesis and whether the dysbiosis of microbiota leads to the onset of IBD or the inflammation triggered by IBD lead to the change of gut microbiota structure. Secondly, gene sequencing revealed that different probiotic strains from the same bacteria species could have wide phylogenetic differences, which means the human body would respond differently to the strains of probiotics from the same bacteria species [72]. However, in many papers, they failed to specify the strains of the probiotics they used by only referring to genus or species level, which might lead to failing attempts in the repeat experiments conducted by a different research group. Thirdly, genetic heterogeneity is another factor that needs to be taken into consideration. We classify two strains of bacteria from the same species if they have a relative ratio of binding of 70% DNA: DNA homology of the genomes at optimal and stringent reassociation temperatures (optimal temperature, 25°C below the melting point of the DNA; stringent temperature, 15°C below the melting point of the DNA) [102]. Due to the technology boundary, so far, we could not further specify the rest 30% of the genomes between these two strains. In other words, these technology boundaries might lead to misclassifying bacterial strains. In designing probiotics, strain specificity is one of the most critical measurements which can directly affect the treatment efficacy of the probiotics. Therefore, more precise phylogenetic analyzing tools need to be investigated to provide a higher specific level.

More importantly, a global standard for naming the bacterial strain needs to be constructed so that avoiding the situation when a single strain has more than 20 different names within the world, which would complex the classification system. Finally, disease specificity is the other vital consideration in designing probiotics. The uses of probiotics are diverse, taking IBD as an example. Some probiotics focus on treating the CD, whereas some focus on UC. Thus, when taking the probiotic treatment, we should narrow it down to the specific subtype of the disease to maximize the efficiency of the probiotics. In this case, personalized probiotics should be suggested. Instead of giving the same probiotics to all the IBD patients, in the future, we should select the best match probiotic or probiotics according to patients' age, eating habits, history of the IBD, types of IBD, etc. For instance, the probiotics prescription of IBD patients who lack daily SCFA intake should contain more *Faecalibacterium prausnitzii* (SCFA-producing bacteria strain) than others.

5. Conclusion

In this review, we have summarized the factors contributing to the incidence of IBD, including nonmicrobiome-related factors such as dietary factors, genetics, host immune system, gender and ethnicity, and microbiome-related factors such as viruses, fungi, and especially bacteria. Based on these internal and external environmental factors, we discussed the preferred direction of microbiome-based treatment in clinical trials, including correcting dysbiosis and controlling inflammation. Each treatment method has its limitation and advantages. Regarding future directions, we have identified some promising areas for addressing these objectives, for example, personalized probiotics, microbial drugs, combination therapy, etc.

Data Availability

No data is available.

Conflicts of Interest

The authors have declared no competing interests.

Authors' Contributions

Jie Wang wrote the manuscript. Liwei Xie edited, proofed, and revised the manuscript. Puxuan Zhang, Shujie Chen, and Huimin Duan participated in the discussion, language editing, and manuscript revision.

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