

Intestinal microecology dysbiosis in inflammatory bowel disease: Pathogenesis and therapeutic strategies

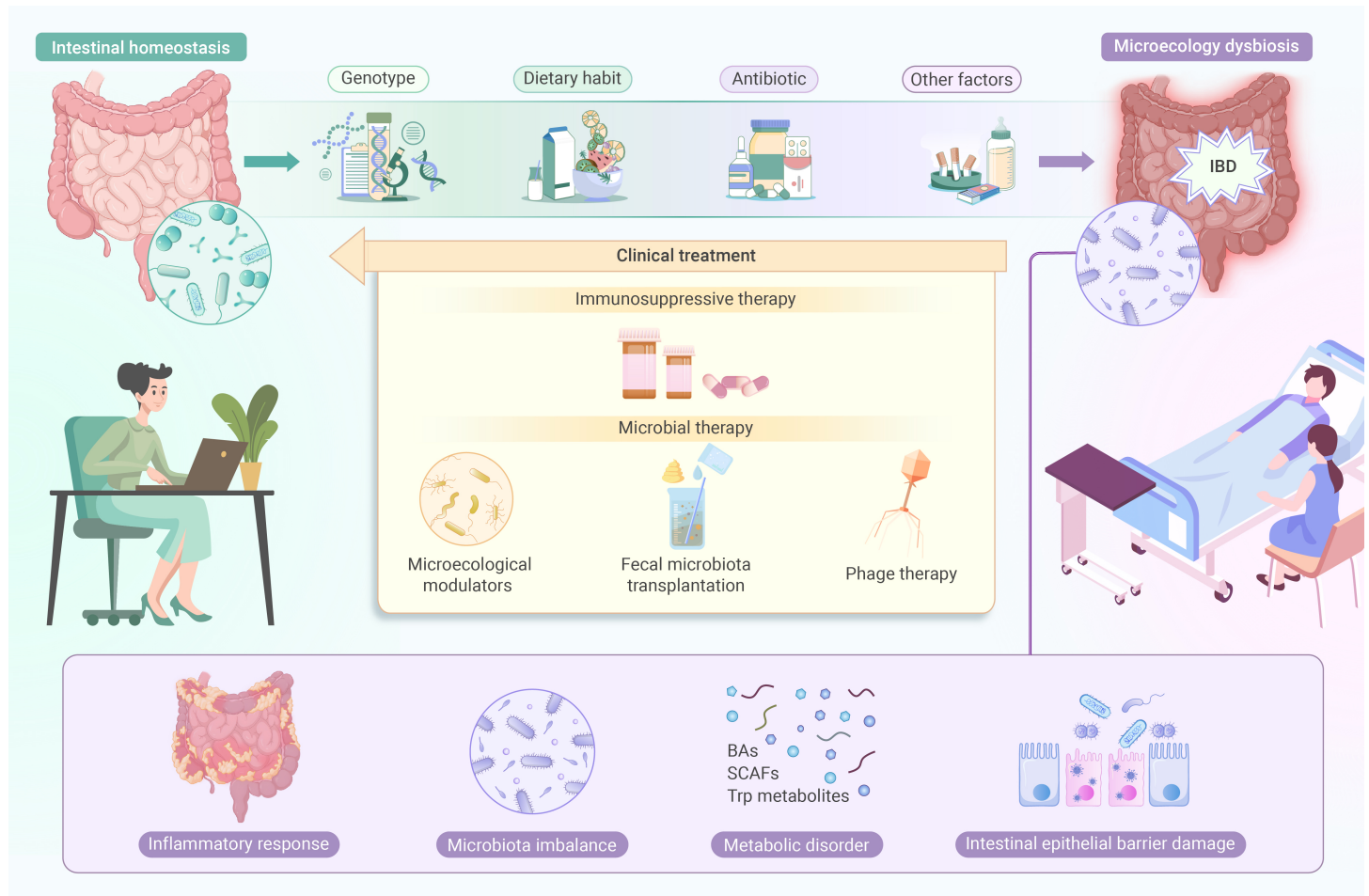
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Intestinal microecology dysbiosis promotes the occurrence and progression of inflammatory bowel disease (IBD).
- Microecology dysbiosis induces intestinal epithelial destruction, chronic inflammation, and metabolic disorders.
- Microbial therapy is a promising emerging strategy for the treatment of IBD.
- Intestinal organoids have advantages in IBD and microecology research.

Intestinal microecology dysbiosis in inflammatory bowel disease: Pathogenesis and therapeutic strategies

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Inflammatory bowel disease (IBD) is an autoimmune disease characterized by non-infectious, chronic, and recurrent inflammation in the intestine, with unclear pathogenesis and increasing incidence. Currently, immunosuppressive drugs are commonly used in the clinical treatment of IBD to alleviate intestinal inflammation, but their effectiveness is not ideal. Therefore, it is crucial to investigate the pathogenesis of IBD and develop new therapeutic strategies. As a significant component of the intestinal barrier, the microbiota is closely related to metabolism, barrier protection, and immune regulation. With advances in research technology, the pivotal role of intestinal microecology dysbiosis in the progression of IBD has gained attention in recent years. Microbial therapies, including microecological modulators, fecal microbiota transplantation, and phage therapy have also become research hotspots in IBD treatment, showing promising applications. However, the complex relationship between intestinal microecology and IBD remains unclear, and clinical trials investigating microbial therapies face limitations. To inspire further research, this review thoroughly summarizes the physiological structure and function of intestinal microecology, as well as the characteristics and pathogenesis of microecology dysbiosis in IBD patients. We also analyze the research status of microbial therapies for IBD and the application of organoid models in preclinical studies.

INTRODUCTION

Inflammatory bowel disease (IBD), mainly comprising ulcerative colitis (UC) and Crohn's disease (CD), is an autoimmune disease characterized by non-infectious, chronic, and recurrent inflammation of the intestine, significantly affecting patients' quality of life.¹ It has become a global burden due to its increasing incidence and prevalence.² However, the pathogenesis of IBD remains unclear, involving genetics, immunity, intestinal microbiota, and environmental factors.³ The clinical treatment for IBD currently focuses on using immunosuppressive medications to reduce chronic intestinal inflammation. However, this approach also raises the risk of infections and cancer in patients.^{4,5} Furthermore, the effectiveness of immunosuppressive therapy tends to decrease over time, with about a third of patients showing no response to the initial treatment.⁶ Therefore, it is urgently necessary to develop new strategies to improve treatment outcomes.

The intestinal microbiota is a crucial structural and functional component of the intestinal barrier, interacting with the host's intestinal environment and playing a vital role in maintaining metabolic homeostasis, barrier integrity, and immune regulation.⁷ In a healthy state, intestinal microbiota constrain and coordinate with each other to maintain the balance of the intestinal microecology. The composition of the intestinal microbiota in IBD patients differs from that of healthy individuals, especially in terms of the richness and diversity of specific bacterial taxa.⁸ Intestinal microecology dysbiosis has gained attention as a possible trigger or consequence of IBD, which also provides new strategies for clinical treatment. Currently, microbial therapies aimed at improving the intestinal microecology of IBD patients, such as microecological modulators, fecal microbiota transplantation (FMT), and phage therapy, have progressed in clinical trials and shown promising results. However, they still face limitations and challenges in translating to routine clinical treatments.^{9,10} To provide a convincing theoretical basis for microbial

therapy, it is crucial to apply and optimize preclinical research models, such as organoids, to further study the mechanisms of interaction between the intestinal microbiota and the host.

This review elaborates on the advancements in IBD pathogenesis from the perspective of intestinal microecology dysbiosis, analyzes the research status of microbial therapies, and summarizes the application of organoids in the study of intestinal microbiota. By gaining a deep understanding of the physiological structure and function of intestinal microecology, along with its dysbiosis characteristics and pathogenic mechanisms in IBD patients, we aim to inspire the development of microbial therapies.

NORMAL INTESTINAL MICROECOLOGY

Composition and location

The healthy intestine contains a diverse range and abundance of microbes, mainly bacteria, viruses, and fungi.¹¹ Bacteria are the most significant component of the intestinal microbiome, comprising around 1000 species. *Firmicutes* are the dominant group (50%–75%), followed by *Bacteroides* (10%–50%), *Actinomycetes* (1%–10%), and *Proteus* (often less than 1%).¹² The human intestinal environment is a diverse ecosystem. (Figure 1) The changes in anatomical location and physiological function of each segment result in gradient differences along the axis from the duodenum to the rectum in various features, including decreased oxygen levels, thickening of the mucus layer, reduced bile acid (BA) secretion, increased pH, reduced secretion of antimicrobial peptides (AMPs), and prolonged gastrointestinal transit time. These factors affect the distribution of intestinal microbiota, increasing their abundance and diversity along the axis.^{13,14} Additionally, the stratification of the intestinal wall's physiological structure influences the radial distribution of non-luminal commensal bacteria. (Figure 1) Although the small and large intestines have significantly different functions, their intestinal wall structures share certain similarities. The layers of the intestine from the lumen outward are mucosal, submucosal, muscular, and serosal. Among them, the mucosal layer can be subdivided into epithelial layer, mucosal lamina propria containing lymphoid tissue, and muscular layer.¹⁵ Only a few bacteria can invade the epithelial and lymphoid tissues within the cellular structure of the intestinal wall, while most bacteria are impeded by AMPs and the mucus layer, thereby preserving intestinal homeostasis.¹⁶ Due to the differences in the secretory functions of the constituent cells in the small intestine and large intestine, there are variations in the composition and structure of the mucus covering the surface of the epithelial cells. The mucus in the small intestine is unstructured, yet it is rich in AMPs secreted by Paneth cells located in the crypts, which serve to inhibit microbial colonization and thus maintain intestinal homeostasis.¹⁷ However, goblet cells in the colonic epithelium can produce mucus that forms a bilayer structure. The inner layer is densely sterile, while the outer layer is populated with mucosa-associated microbes, crucial for the intestinal barrier.^{18,19} In addition to bacteria, the intestine also provides a conducive environment for fungi and viruses. There are more than 50 genera of intestinal fungi dominated by *Candida*, *Saccharomyces*, and *Cladosporium* species. Viruses are highly diverse, including prokaryoviruses (bacteriophages) and eukaryoviruses, which interact with bacteria and fungi to maintain the intestinal microecology.^{20,21}

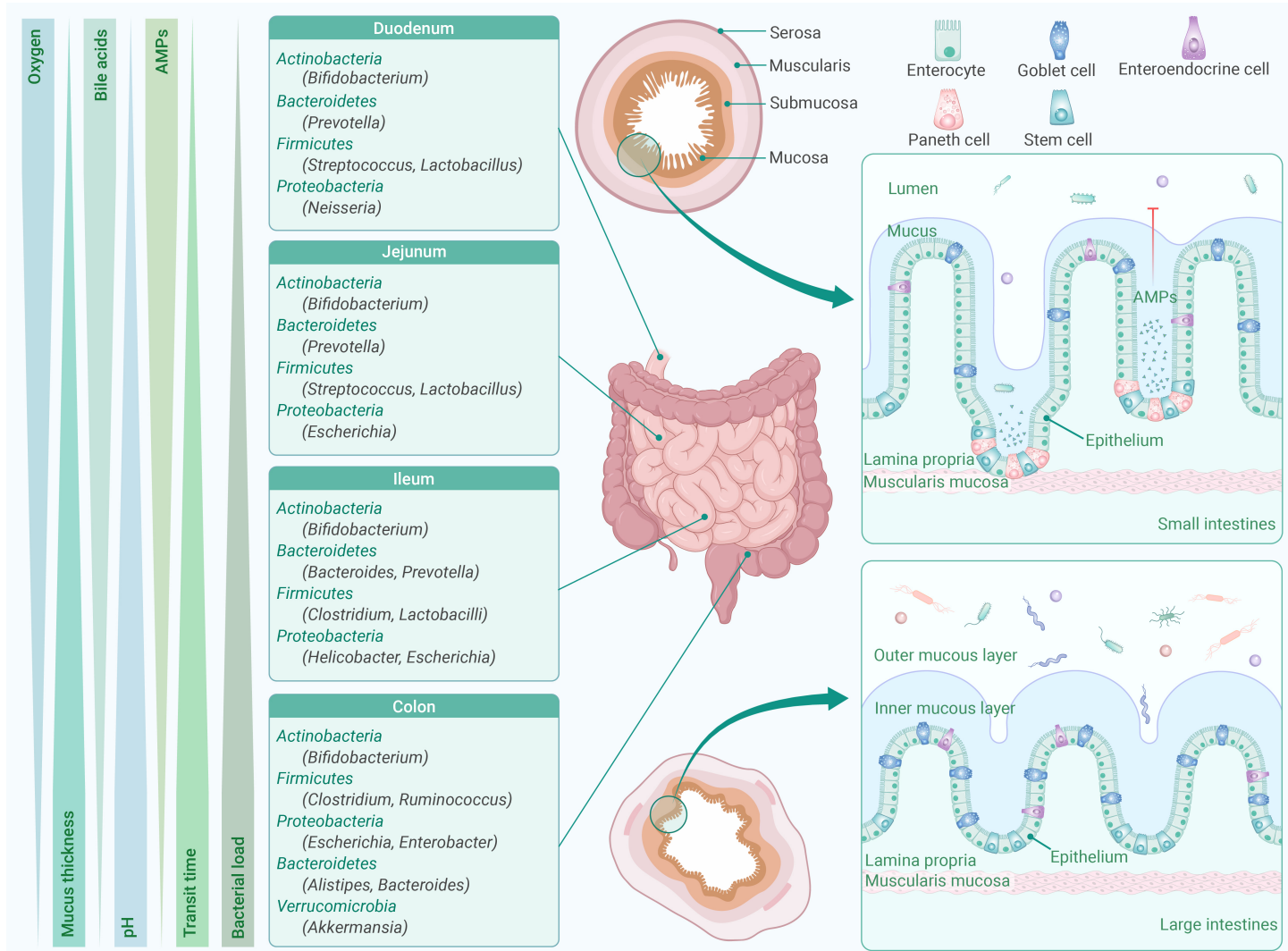


Figure 1. Intestinal structure and distribution of bacteria.

Function and mechanism

As the main component of the biological barrier, intestinal microecology directly inhibits the colonization of pathogens. Establishing colonization resistance primarily depends on the competitive interactions between symbiotic bacteria and pathogens, including nutrient and spatial competition. Symbiotic bacteria, leveraging their numerical advantage, can occupy a significant amount of physical space and consume various essential molecules, including organic acids, amino acids, sugars, and metals, as well as respiratory electron acceptors such as O_2 and NO_3^- .²² This competitive behavior limits the available sites for pathogen adherence and reduces the availability of essential nutrients and molecules, thereby establishing colonization resistance.²² Bacteriostatic or bactericidal ability is also critical for resistance to colonization by specific pathogens. Bacteria produce a diverse class of peptides known as bacteriocins, which have specific inhibitory or killing effects on similar symbiotic bacteria and pathogens.²³ For instance, bacteriocins produced by *Escherichia coli* directly inhibit the growth of enterohaemorrhagic *E. coli* (EHEC).²⁴ Furthermore, bacteriocins produced by intestinal commensals, primarily probiotics such as bifidobacteria and lactic acid bacteria, have been shown to inhibit pathogens, including *Listeria monocytogenes*, *Salmonella* species, *Clostridium botulinum*, *C. perfringens*, and *C. difficile*.^{25,26} In addition to producing bacteriocins, symbiotic bacteria can resist pathogenic invasion through the type VI secretion system (T6SS). T6SS is a protein translocation complex found in certain gram-negative bacteria, including three predominant genera: *Bacteroides*, *Parabacteroides*, and *Prevotella*.²⁷ Through the T6SS, Gram-negative bacteria detect non-native

symbiotic bacteria and release antibacterial effectors that target essential components of bacterial cells, such as peptidoglycan, membrane phospholipids, nucleic acids, nicotinamide adenine dinucleotide, adenosine triphosphate, and cell division proteins, into the target bacterial cytosol or periplasm, thus inhibiting the growth and colonization of pathogens.^{28,29} In addition to forming the biological barrier, the microbiota maintains intestinal homeostasis by regulating metabolism, stabilizing the intestinal epithelial barrier (IEB), and modulating the immune system (Table 1).

Influencing factors

Since intestinal microecology involves complex physiological functions, maintaining its homeostasis is crucial. Studies have demonstrated that several factors, such as genetics, diet, and drug use, influence intestinal microecology. The host's genotype can influence the type and abundance of intestinal microbiota.³⁰ Specific genes associated with IBD susceptibility have been found to disrupt the balance of the intestinal microbiota. *NOD2* encodes an intracellular receptor in intestinal epithelial cells (IECs) that can interact with peptidoglycan from bacteria, thereby preventing pathogenic bacterial colonization.³¹ *Nod2*-knockout mice have reduced levels of antimicrobial defensins and a higher bacterial load compared to wild-type mice, making them more susceptible to colitis.³² *ATG16L1* regulates autophagy, a cellular pathway responsible for the lysosomal breakdown and clearance of intracellular bacteria. Its mutations hinder bacterial clearance and antigen presentation, affecting the intestinal microbiota, such as increasing *Bacteroides* species.³³ Dectin-1, encoded by *CLEC7A*, detects β -1,3-glucans in fungal cell

Table 1. Mechanisms of microbial involvement in intestinal physiological functions

Physiological functions		Microbes	Mechanisms	References
Metabolism	Breakdown	<i>Bacteroidetes</i> and <i>Firmicutes</i>	Facilitate the breakdown of resistant starch and indigestible carbohydrates.	243,244
		<i>Bacteroides</i>	Participates in carbohydrate metabolism through degrading glycosyl transferases, glycoside hydrolases, and polysaccharide lyases.	
		<i>B. thetaiotaomicron</i>	Enhances lipid hydrolysis efficiency by upregulating the expression of colipase.	
		<i>Lactobacillales</i>	Participates in amino acid metabolism mainly through arginine deiminase pathway.	
	Synthesis	<i>Bacteroidetes</i> Lactic acid bacteria	Participates in thiamine biosynthesis and transport. Produces riboflavin and folates.	245 246 247
IEB maintenance	Mechanical	<i>Faecalibacterium prausnitzii</i> and <i>Eubacterium rectale/Roseburia</i>	Inhibits permeability-promoting claudin-2 tight-junction protein expression by producing butyrate.	248,249
		<i>Akkermansia muciniphila</i>	Adheres to the intestinal epithelium and enhances the integrity of the enterocyte monolayer.	250
		<i>B. thetaiotaomicron</i>	Maintains desmosomes at the epithelial villus by inducing the expression of sprr2A.	251
		<i>Bifidobacterium bifidum</i>	Enhances the intestinal epithelial tight junction barrier by targeting the TLR2 pathway in an NF- κ B-independent manner.	252
	Chemical	<i>B. thetaiotaomicron</i> and <i>F. prausnitzii</i>	Modifies goblet cells and mucin glycosylation by metabolically complementary.	253
		<i>Lactobacillus</i>	Promotes intestinal mucus formation and homeostasis through producing L-Ornithine.	254
		<i>Clostridia</i> clusters VI and XIVa	Promote Tregs accumulation in normal mucosa by inducing Treg-inducing factors, such as TGF- β and IL-10, released from IECs.	255
Immune regulation		<i>B. fragilis</i>	Stimulates immunologic development and induces the production and function of Tregs by secreting the immune modulator PSA.	256
		<i>L. murinus</i>	Promotes Tregs differentiation by inducing TGF- β and IL-10 expression in DCs and macrophages to improve intestinal inflammation.	257
		<i>L. reuteri</i>	Suppresses TNFs production through the modulation of PKA and ERK signaling by converting L-histidine into histamine.	258
		T4 phages	Inhibits infection-induced ROS and NF- κ B activation.	259
		<i>S. boulardii</i>	Diminishes pro-inflammatory responses and induces mucosal anti-inflammatory signaling.	260

Abbreviations: DC, dendritic cell; ERK, extracellular signal-regulated kinase; IEB, intestinal epithelial barrier; IECs, intestinal epithelial cells; IL, Interleukin; NF- κ B, nuclear factor-kappa B; PKA, protein kinase A; PSA, polysaccharide A; ROS, reactive oxygen species.; Sprr2A, proline-rich small protein 2A; TGF, transforming growth factor; TLR, Toll-like receptor; Tregs, regulatory T cells.

walls and activates intracellular signaling through domain-containing protein 9, a protein encoded by *CARD9*, to initiate an immune response against fungi. Mutations in *CLEC7A* or *CARD9* lead to an increased abundance of fungal species associated with IBD-associated dysbiosis.^{34,35} Additionally, a missense mutation in *SLC39A8*, which encodes a zinc and manganese transporter, is associated with an increased incidence of CD and altered microbiota composition.³⁶ Food, as a substrate for microbial fermentation, is the most direct factor affecting intestinal microecology.³⁷ The higher prevalence of IBD in Western countries may be related to dietary habits. The Western diet, characterized by high levels of sugar, fat, and protein, along with a low fiber content, may encourage the growth of pathogenic bacteria in the intestine. This can lead to microbial dysbiosis, thereby increasing the risk of developing IBD.³⁸ Antibiotic treatment also disrupts the normal and healthy balance of the intestinal microbiota, leading to the loss or inhibition of beneficial species while encouraging the growth and proliferation of potentially pathogenic or harmful bacteria.³⁹ Studies have shown that using antibiotics early in life increases the risk of IBD.⁴⁰ Moreover, other risk factors such as smoking, exposure to gastroenteritis, and breastfeeding can also affect the balance of the intestinal microbiota.⁴¹

INTESTINAL MICROECOLOGY DYSBIOSIS IN IBD

Microbiota imbalance

The intestinal microbiota of IBD patients significantly differs from that of

healthy individuals. (Table 2) Although the causal relationship between intestinal microbiota dysbiosis and IBD is not fully understood, abnormal microbiota affects intestinal homeostasis, thereby driving IBD progression. Therefore, understanding the microbial composition of IBD can offer new strategies for clinical diagnosis and treatment. By analyzing microbes in mucosa or feces, several studies have shown that IBD patients have lower numbers and diversity of intestinal bacteria compared to healthy individuals. This includes a decreased abundance of bacteria with anti-inflammatory properties, such as *Clostridium* groups IV and XIVa, *Bacteroides*, *Suterella*, *Roseburia*, *Bifidobacterium* species, and *F. prausnitzii*.⁴² On the contrary, some pathogens, such as *Proteobacteria* members (including invasive and adherent *E. coli*), *Fusobacterium* species, and *Ruminococcus gnavus*, are increased.⁴³ In contrast to bacteria with reduced diversity, the fungal diversity and abundance in the feces and mucosa of IBD patients, specifically *Candida* and *Malassezia* species, increase.⁴⁴ Additionally, the loss of core phages and the increased abundance of lysogenic phages in IBD are closely associated with the imbalance of intestinal bacteria.^{45,46} Eukaryotic viruses are also altered, contributing to intestinal inflammation.²¹ While the general trends in the changes of intestinal microbiota in IBD patients are consistent, there are variations in the composition of the microbiota among individuals and across different regions of the intestines. Studies have shown that microbial dysbiosis is more pronounced in CD than in UC, with a lower diversity, more altered composition, and a less stable community.⁴⁷ There are also differences in the

Table 2. Abnormal changes in the intestinal microbiota of IBD patients

Types	Increased	Decreased	References
Bacteria	UC Ÿ Phylum: <i>Proteobacteria</i> Ÿ Genus: <i>Collinsella</i> and <i>Fusobacterium</i> Ÿ Species: <i>E. coli</i>	Ÿ Phylum: <i>Firmicutes</i> and <i>Bacteroidetes</i> Ÿ Cluster: <i>Clostridium</i> XIVa and IV Ÿ Species: <i>F. prausnitzii</i> , <i>R. hominis</i> , <i>A. muciniphila</i> and <i>Butyrivibrio</i> <i>pulliaecorum</i>	42,47,261
	CD Ÿ Phylum: <i>Proteobacteria</i> and <i>Fusobacteria</i> Ÿ Family: <i>Bacteroidaceae</i> , <i>Enterobacteriaceae</i> , <i>Pasturellaceae</i> , <i>Veillonellaceae</i> , <i>Neisseriaceae</i> and <i>Fusobacteriaceae</i> Ÿ Genus: <i>Escherichia</i> , <i>Fusobacterium</i> , <i>Atopobium</i> , and <i>Enterobacter</i> Ÿ Species: <i>E. coli</i> and <i>R. gnavus</i>	Ÿ Order: <i>Erysipelotrichales</i> and <i>Clostridiales</i> Ÿ Family: <i>Peptostreptococcaceae</i> , <i>Christensenellaceae</i> , <i>Lachnospiraceae</i> , <i>Ruminococcaceae</i> and <i>Actinomycetaceae</i> Ÿ Genus: <i>Coprococcus</i> , <i>Bacteroides</i> , <i>Blautia</i> , <i>Ruminococcus</i> , <i>Anaerostipes</i> , <i>Methanobrevibacter</i> , <i>Lachnospira</i> , <i>Roseburia</i> , <i>Bifidobacterium</i> , <i>Prevotella</i> , <i>Dorea</i> and <i>Oscillospira</i> Ÿ Cluster: <i>Clostridium</i> XIVa and IV Ÿ Species: <i>C. aerofaciens</i> , <i>F. prausnitzii</i> and <i>Helicobacter pylori</i>	42,47,262,263
	IBD Ÿ Genus: <i>Streptococcus</i> , <i>Veillonella</i> , <i>Peptostreptococcus</i> , <i>Campylobacter</i> and <i>Enterococcus</i>	Ÿ Genus: <i>Suterella</i> , <i>Flavobacterium</i> and <i>Eubacterium</i>	43,264
Fungi	UC Ÿ Genus: <i>Wickerhamomyces</i> , <i>Aspergillus</i> , <i>Sterigmatomyces</i> and <i>Candida</i>	Ÿ Genus: <i>Exophiala</i> , <i>Alternaria</i> , <i>Emericella</i> , <i>Epicoccum</i> , <i>Acremonium</i> , <i>Trametes</i> and <i>Penicillium</i>	265
	CD Ÿ Family: <i>Cystofilobasidiaceae</i> Ÿ Genus: <i>Candida</i> , <i>Dioszegia</i> , <i>Debaryomyces</i> and <i>Malassezia</i> Ÿ Species: <i>G. moniliformis</i> , <i>A. brassicicola</i> and <i>C. neoformans</i>	Ÿ Genus: <i>Leptosphaeria</i> and <i>Trichosporon</i> Ÿ Species: <i>S. cerevisiae</i> , <i>Neurospora tetrasperma</i> , <i>M. restricta</i> , <i>Moniliophthora perniciosa</i> and <i>Nicotophodium gansuense</i>	44,51,266,267
	IBD Ÿ Species: <i>Clavospora lusitanae</i> , <i>Cyberlindnera jadinii</i> and <i>Kluyveromyces marxianus</i>	Ÿ Species: <i>S. pastorianus</i> and <i>S. bayanus</i>	264,268
Viruses	UC Ÿ Prokaryotic: <i>Escherichia</i> and <i>Enterococcus</i> phages Ÿ Eukaryotic: <i>Hepadnaviridae</i> and <i>Pneumoviridae</i>	Ÿ Eukaryotic: <i>Polydnaviridae</i> , <i>Tymoviridae</i> and <i>Anelloviridae</i>	21,186,269
	CD Ÿ Eukaryotic: <i>Baculoviridae</i>	Ÿ Eukaryotic: <i>Virgaviridae</i> , <i>Artitiviridae</i> and <i>Bromoviridae</i>	21
	IBD Ÿ Prokaryotic: <i>Lactococcus</i> , <i>Lactobacillus</i> , <i>Clostridium</i> and <i>Streptococcus</i> phages Ÿ Eukaryotic: <i>Herpesviridae</i>	Ÿ Prokaryotic: <i>Bacteroides</i> phages (<i>crAssphage</i>) and <i>Microviridae</i> phages	46,186,270

Abbreviations: CD, Crohn's disease; IBD, Inflammatory bowel disease; UC, ulcerative colitis.

predominant intestinal microbes of IBD patients across different disease activity indexes and stages of disease course.^{48,49} In UC patients experiencing dysregulation of the entire intestinal microbiota, variations in inflammatory status across different intestinal regions also influence the local bacterial distribution.⁵⁰ Compared to uninflamed mucosa from CD patients, *Candida* species, *Gibberella moniliformis*, *Alternaria brassicicola*, *Cryptococcus neoformans*, and *Baculoviridae* are significantly more prevalent in actively inflamed mucous membranes.^{21,51} The investigation of microbial dysbiosis in IBD still presents numerous controversies and unknowns, and further exploration is imperative. Microbial dysbiosis in IBD disrupts the biological barrier, thus weakening resistance to pathogens. It also affects intestinal homeostasis by impacting the IEB, immune system, and metabolism.

Intestinal epithelial barrier damage

Specialized IECs of various isoforms are interconnected through apical junction complexes that include tight junctions (TJs), adherens junctions, and desmosomes. This interconnection forms the basis of the intestinal mechanical barrier.⁵² As a physical demarcation between the luminal contents and the underlying immune system, it ensures the impermeability of the IEB and serves a protective role against pathogens. In the physiological state, commensal microbiota protects the intestinal barrier by maintaining TJ integrity. However, in IBD patients, certain pathogen levels can abnormally increase and disrupt the junctional complex by expressing effector proteins.⁵³ For example, enteropathogenic *E. coli* disrupts apicobasal polarity complexes by secreting the effectors EspF and Map, thus altering actin dynamics and disrupting TJ structure and function.⁵⁴ The decreased expression of TJ proteins (TJPs) compromises the IEB and facilitates the penetration of pathogens, while the remodeling of TJs can alleviate IBD.⁵⁵ Besides forming a physical barrier through TJs, IECs form a mucus layer on the lumen surface

of the intestinal mucosa by secreting mucus and AMPs such as protein hydrolases, glycolytic enzymes, and AMPs, serving as the first line of defense against pathogens invasion.¹² However, IBD patients show a notable rise in mucus-degrading bacteria, such as *R. gnavus* and *R. torques*, which dissolve mucus, thus increasing intestinal translocation.⁵⁶ Enteric pathogens such as *Salmonella*, *Streptococcus*, and *Staphylococci* can evade the bactericidal activity of AMPs. They repel, intercept, efflux, and destroy AMPs through cell surface modification, competitive binding, removal or secretion, and proteolytic inactivation.⁵⁷⁻⁵⁹ The thinning of the mucus layer and reduced resistance to AMPs increase the exposure of IECs in IBD patients. As pathogens accumulate, they release pathogenic factors that directly attack intestinal cells, leading to IEB damage.^{60,61} Studies have shown that probiotics can improve IEB dysfunction and alleviate intestinal inflammation. Administering VSL#3, a probiotic containing eight strains of bacteria, to mice with dextran sulfate sodium (DSS)-induced colitis helps prevent the decrease in TJPs expression and reduces cell apoptosis.⁶² Furthermore, VSL#3 promotes the secretion of colonic mucin (MUC), thereby improving IEB function in Muc2 mucin-deficient mice.^{63,64}

Immune activation

IBD is an autoimmune disease, and abnormal immune activation plays a significant role in its pathogenesis. In IBD patients, pathogens may penetrate the intestinal mucus layer and mechanical barrier, triggering immune responses that can lead to chronic inflammation. Under inflammatory conditions, pathogens gain a growth advantage over symbiotic bacteria, further exacerbating intestinal microecology dysbiosis.⁶⁵

Microbial identification. IECs and lamina propria immune cells detect abnormal microbiota and trigger inflammatory responses through pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), nucleotide-

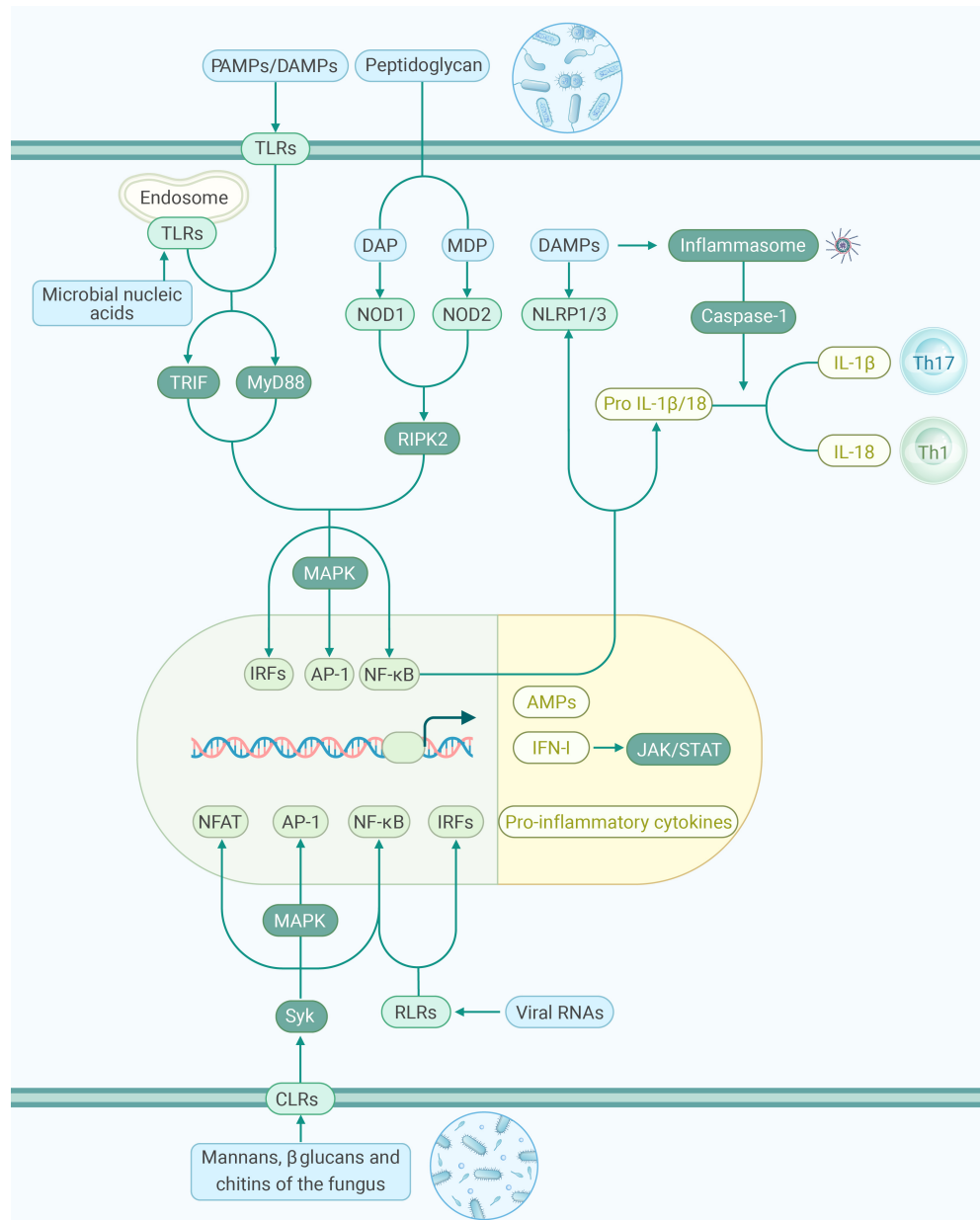


Figure 2. Mechanism by which receptors recognize microbiota.

scription factors such as nuclear factor-kappa B (NF- κ B), activator protein-1 (AP-1), and interferon regulatory factor (IRF) leading to the synthesis of type I (IFN- I) and pro-inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)- α .⁶⁹ Additionally, NF- κ B, activated by TLRs, promotes the transcription of NLRP3, proIL-1 β , and proIL-18, thus facilitating the assembly and function of the inflammasome.⁷¹ To prevent pathogenic inflammation, TLR signaling is regulated by multiple layers of control mechanisms, such as receptor folding, intracellular trafficking, post-translational modifications, ligand acquisition and transport, and downstream signaling propagation.⁶⁷ In vitro data suggest that the surface receptors TLR2 and TLR4 on IECs show low reactivity to ligands.^{72,73} Under physiological conditions, antigen-presenting cells in the innate layer also exhibit limited PRR expression and show significant inflammatory energy, despite their strong phagocytic and bactericidal capabilities.⁷⁴ TLRs on the basolateral side of the epithelium or within the cytoplasm are unable to detect extracellular symbionts under physiological conditions. They are activated only when flagellin translocates to the lamina propria or when pathogens inject effector proteins into the cell.^{75,76}

NLRs are also present in the cytoplasmic compartments of IECs and immune cells. D-glutamyl-meso-diaminopimelic acid (DAP) and muramyl dipeptide (MDP), derived from the decomposition of peptidoglycan, can be detected by NOD1 and NOD2, which belong to the NLRC subtypes, respectively.⁷⁷ The activated NOD receptors oligomerize and recruit receptor-interacting protein kinase 2 (RIPK2), thus activating mitogen-activated protein kinase (MAPK), NF- κ B, and IRF pathways,

which promote the expression of pro-inflammatory cytokines, AMPs, and IFN-I.^{77,78} The NLRP1 and NLRP3 subtypes can detect signals produced by bacterial and viral infections in cells, such as lethal factors, mitochondrial DNA, ROS, and potassium efflux. Additionally, they form inflammasomes, which can process and release mature IL-1 β and IL-18 by activating caspase-1.^{78,79} IL-1 β is increased in the mucosa of IBD patients and is associated with the proliferation of pathogenic helper T (Th)17 cells.⁸⁰ IL-18 is increased in the serum of IBD patients, which may be pathogenic in IBD by promoting IFN- γ production in Th1 cells.⁸¹

As transmembrane receptors, CLRs detect carbohydrate ligands that are mainly found on the surface of fungal cells, including mannans, β -glucans, and chitins.⁸² Activated CLRs ultimately activate transcription factors, such as NF- κ B, nuclear factor of activated T cells (NFAT), and AP-1, mainly through Syk-dependent pathways.⁸³ They trigger pro-inflammatory immune responses, such as phagocytosis, oxidative stress, and cytokine production.⁸⁴ Excessive activation of CLRs promotes the progression of IBD when there is an abnormal increase in intestinal fungi.

RLRs are a family of RNA helicases that detect viral RNAs and induce IFN-I expression and secretion by activating IRF and NF- κ B.⁸⁵ IFN-I is secreted and activate IFN receptors in an autocrine and paracrine manner, subsequently inducing the expression of hundreds of IFN-stimulated genes through the downstream JAK-STAT signaling cascade to combat viral infections. IFN-

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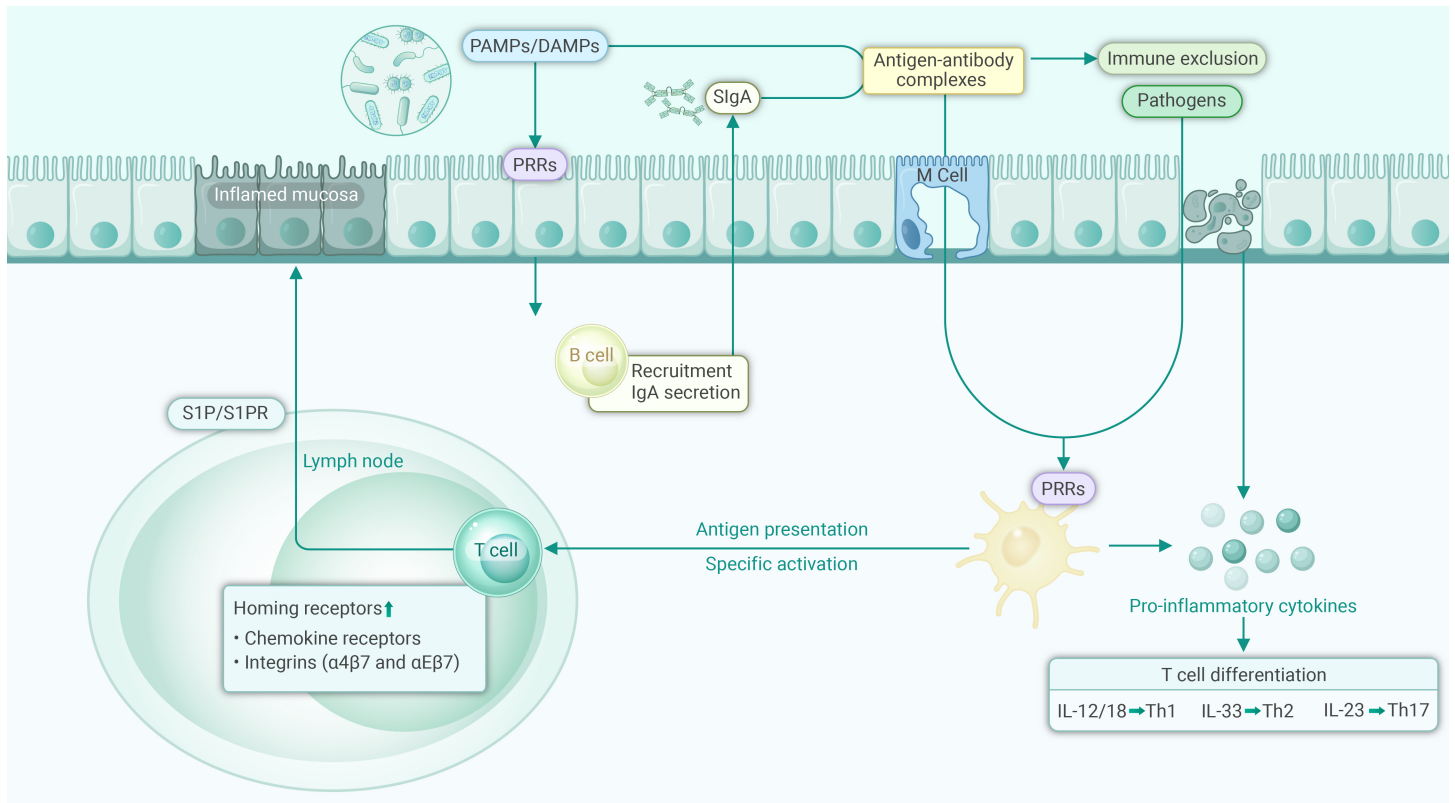


Figure 3. Immune response triggered by intestinal microecology dysbiosis.

I also stimulates adaptive immunity by attracting immune cells and enhancing their function.⁸⁶ In the absence of infection, RIG-I is inactive. When RIG-I patrols its cellular environment, it binds to host RNA and quickly releases it. Eventually, it encounters viral RNA containing specific molecular determinants during an infection, forming a stable signal-active conformation.⁸⁵

Inflammatory response. After PRRs detect the pathogens that breach the IEB, they trigger an excessive immune response in the epithelium and lamina propria, which may contribute to the progression of IBD.⁸⁷ (Figure 3) Upon detecting pathogen invasion, TLR signaling in IECs facilitates the recruitment of B cells and the luminal secretion of Immunoglobulin A (IgA).⁸⁸ The complexes formed by secretory IgA (SIgA) binding to antigens can be trapped in the lumen and expelled or cleared.⁸⁹ In addition to undergoing immune exclusion, the complexes can bind to receptors on microfold cells and be transferred to the lamina propria.^{90,91} These complexes, along with certain pathogens that directly traverse the damaged IEB to reach the lamina propria, can activate dendritic cells (DCs).⁹² In healthy mucosa, intestinal DCs induce immune tolerance. However, in IBD patients, these cells show a significant increase in PRR expression and enhanced activation, promoting adaptive immunity.^{93,94} Naïve T cells are specifically activated in gut-associated lymphoid tissues or mesenteric lymph nodes by antigen-presenting DCs and subsequently upregulate homing receptors, including chemokine receptors and integrins such as α4β7 and αEβ7.⁹² They exit lymph nodes under the guidance of the sphingosine-1 phosphate receptor (S1PR) family and migrate to the inflamed mucosal surface to exert their immune effects.⁹⁵ In addition to inducing adaptive immune responses, DCs, macrophages, and IECs secrete numerous pro-inflammatory cytokines, including IL-1β, IL-6, IL-18, IL-23, and TNF-α.⁹⁶ Activated T cells differentiate into various subpopulations under the influence of cytokines. Th1 cells, induced by IL-12 and IL-18, secrete IFN-γ, TNF-α, and IL-2 to recruit macrophages, natural killer (NK) cells, and CD8⁺ T cells, which are associated with IEB disruption and intestinal inflammation.^{96,97} IL-33, released by damaged IECs, induces Th2 responses characterized by the secretion of IL-4, IL-5, and IL-13. The persistence of the Th2 effector pathway may be associated with chronic inflammation in UC and may induce intestinal mucosal and transmural fibrosis in IBD.⁹⁸ IL-23-induced Th17 cells mediate inflammation by secreting IL-6, IL-17, IL-21, IL-

22, TNF-α, and IFN-γ. Th17 cells and related pro-inflammatory cytokines are significantly upregulated in the intestinal mucosa and lamina propria of IBD patients.^{99,100} The persistent activation of immune cells and the release of pro-inflammatory cytokines, caused by abnormal intestinal microbiota, lead to inflammatory overload. This destroys the IEB and exacerbates pathogen invasion into the intestine.

In addition to the pro-inflammatory response resulting from excessive PRR activation, several studies have demonstrated that the absence of PRRs can contribute to intestinal damage and inflammation by impairing the identification and clearance of pathogen invasion. Epithelial-specific deletion of TLR5 results in decreased anti-flagellin antibody levels and changes the composition and localization of the microbiota, ultimately causing low-grade chronic inflammation.¹⁰¹ NOD2 dysfunction leads to reduced levels of antimicrobial α-defensins and an increased susceptibility to intestinal inflammation.¹⁰² A deficiency in Dectin-3, a myeloid cell-specific CLR family member, impairs the phagocytic and fungicidal abilities of macrophages, thereby promoting the development of colitis.¹⁰³

Both the absence and excessive activation of PRRs affect the immune response, thus disrupting intestinal homeostasis and impacting the intestinal microbiota. Intestinal microecology dysbiosis, in turn, exacerbates the inflammatory response mediated by PRR abnormalities and promotes the development of IBD. Due to individual differences in PRR expression, intestinal microbiota composition, and types of infected cells, PRRs seem to play a dual role in inflammation. To optimize clinical treatment strategies, further research is needed to investigate the interaction between intestinal microecology dysbiosis and PRRs.

Metabolic disorder

Some metabolites, including BAs, tryptophan and its metabolites, and short-chain fatty acids (SCFAs), produced in the intestine with the involvement of the microbiota, also impact intestinal homeostasis. Intestinal microbial dysbiosis can lead to metabolic disorders that promote IBD progression by disrupting the integrity of the intestinal barrier and inducing an inflammatory response. (Figure 4)

Bile acids. Primary bile acids (PBAs) are synthesized in the liver and

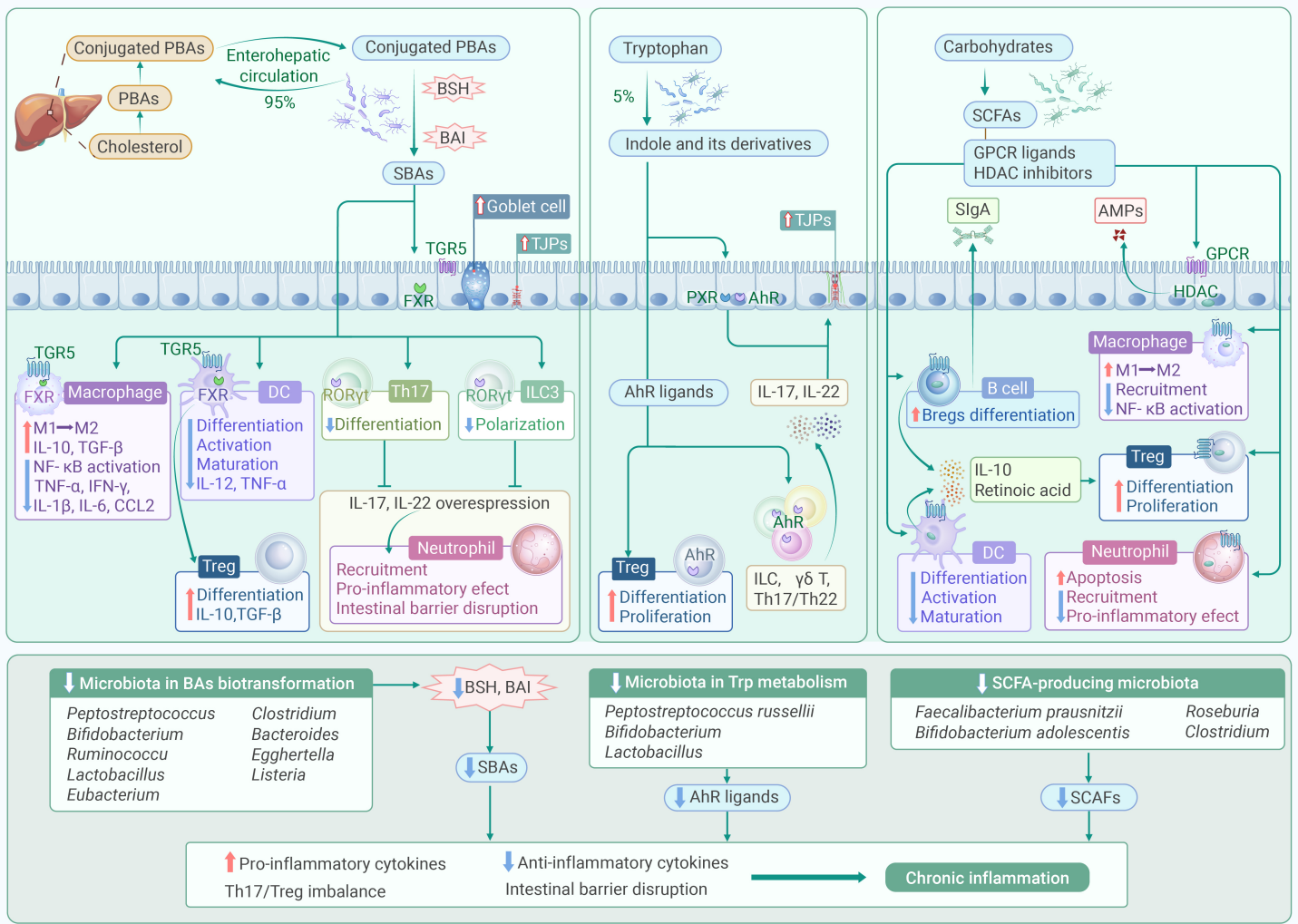
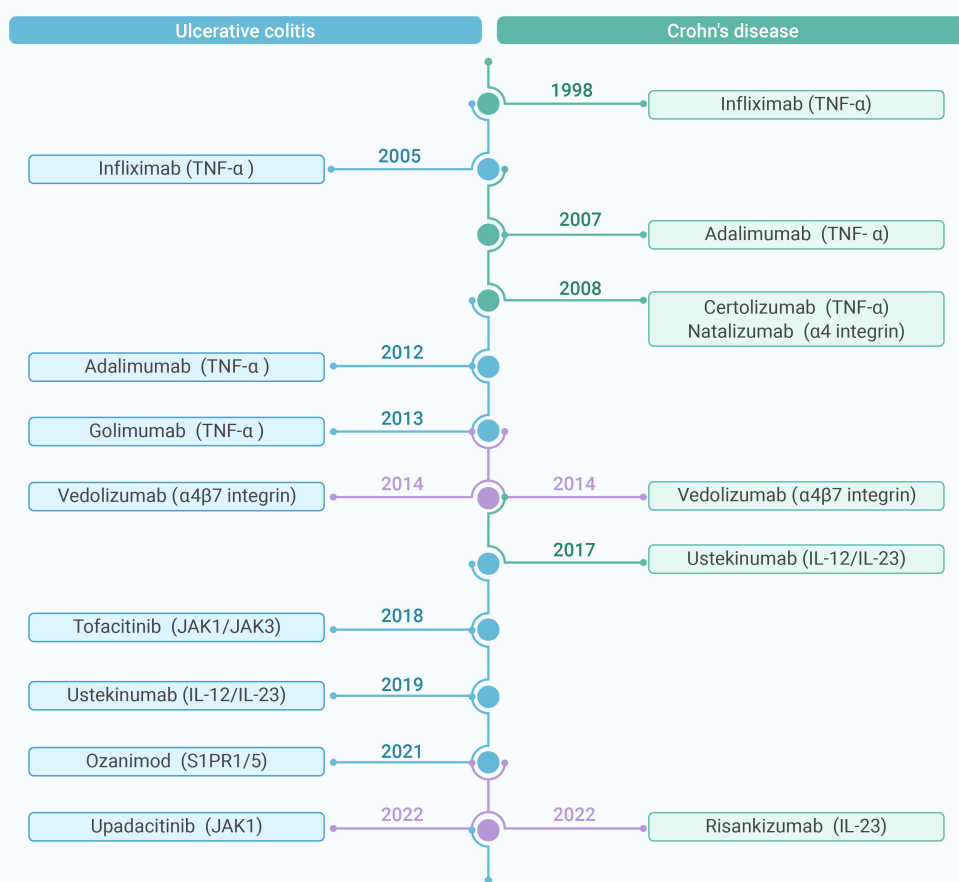


Figure 4. Microbiota-mediated metabolism regulates intestinal homeostasis.

combined with either glycine or taurine to form conjugated PBAs. They are stored in the gallbladder and released into the intestine postprandially, of which about 95% are reabsorbed through the enterohepatic circulation in the terminal ileum. At the same time, the remaining BAs undergo biotransformation mediated by the intestinal microbiota, mainly involving deconjugation, 7 α -dehydroxylation, oxidation, and epimerization, resulting in the production of secondary BAs (SBAs).¹⁰⁴ The enzymes bile salt hydrolase (BSH) and BA-inducible (BAI), produced by the intestinal microbiota, are essential for these biotransformations.¹⁰⁵ BAs, especially SBAs, are involved in regulating metabolic and immune processes by activating various receptors, such as the Farnesoid-X-receptor (FXR) and Takeda G-protein-coupled receptor 5 (TGR5), which are primarily found in IECs, macrophages, and DCs.¹⁰⁶ FXR activation in macrophages inhibits the production of NF- κ B-dependent pro-inflammatory cytokines.¹⁰⁷ TGR5 activation transforms macrophages treated with LPS from M1 to M2 phenotype. It reduces the expression of several pro-inflammatory cytokines, including TNF- α , IFN- γ , IL-1 β , IL-6, and CCL2, and increases the production of anti-inflammatory cytokines such as IL-10 and TGF- β .¹⁰⁸ Moreover, FXR and TGR5 activation can inhibit the differentiation, activation, and maturation of intestinal DCs and downregulate the expression of IL-12 and TNF- α .¹⁰⁹ β -hydroxydeoxycholic acid can promote the generation of Tregs through interactions with FXR in DCs.¹¹⁰ In addition to modulating the immune response, FXR and TGR5 activation can also preserve the intestinal barrier by reducing the loss of goblet cells and upregulating the expression of TJPs.^{111,112} In IBD patients, decreased levels of SBAs, due to a reduction in the number of intestinal microbiota involved in BA biotransformation, affect the activation of BA receptors. This leads to an imbalance between Tregs and Th17 cells, upregulation of pro-inflammatory cytokines,

and disruption of the IEB.¹¹³ Furthermore, SBAs can directly impact pathogenic bacteria. Studies have shown that they inhibit spore germination, growth, and toxin activity of *C. difficile*, which may contribute to the significantly increased risk of *C. difficile* infection in IBD patients.^{114,115}

Tryptophan metabolism. Tryptophan is an essential aromatic amino acid in the human body, playing a vital role as a synthetic precursor for various biologically active substances. Approximately 5% of ingested tryptophan is directly metabolized by the intestinal microbiota to produce indole and its derivatives.¹¹⁶ Indole has been shown to enhance IEB function by strengthening the TJs between IECs by activating the pregnane X receptor (PXR).¹¹⁷ Many indole derivatives exert significant immunomodulatory effects by acting as ligands for the aryl hydrocarbon receptor (AhR).¹¹⁸ In a healthy state, AhR activation can promote innate lymphoid cells (ILCs), Th17/Th22 cells, and intraepithelial lymphocyte $\gamma\delta$ T cells to produce IL-17 and IL-22, which help maintain and protect the intestinal barrier function by promoting IEC proliferation, regulating TJPs, and promoting the secretion of mucins and AMPs.^{119,120} However, in IBD patients, IL-17 and IL-22 are overexpressed due to the constant invasion of the intestinal epithelium by a large number of pathogens. These pathogens recruit neutrophils to excessively secrete pro-inflammatory cytokines and damage the IEB, thereby exacerbating intestinal inflammation.¹²⁰ Studies have shown that SBAs, including 3-oxolithocholic acid and isolithocholic acid, can act as inverse agonists of retinoid-related orphan receptor (ROR)yt in Th17 and ILC3 cells, inhibiting their polarization and reducing IL-17 production to reduce intestinal inflammation.^{121,122} AhR activation also induces Foxp3 expression and promotes the differentiation and proliferation of Tregs.¹²³ In addition to immunomodulation, AhR-mediated indole derivatives can prevent the increase in intestinal permeability associated with mouse models of colitis by maintaining the integrity of the

Figure 5. Biologics and novel small molecule drugs for IBD.

microbiota imbalance, IBD is commonly treated with immunosuppressive drugs in clinical practice. Inhibiting the inflammatory response can directly alleviate tissue damage, as well as help reshape the microecology and enhance intestinal barrier function. Conventional drugs for IBD treatment, such as aminosalicylates, glucocorticoids, and immunomodulators (azathioprine, 6-mercaptopurine, cyclosporine, methotrexate), have non-specific immunosuppressive effects. They induce and maintain IBD remission through various anti-inflammatory and immunomodulatory functions, including inhibiting pro-inflammatory cytokine production and lymphocyte proliferation.¹³⁸ In the 1990s, the introduction of infliximab, which targets TNF- α , marked the beginning of the biologics era in IBD treatment. Compared to traditional therapy, biologics that act on specific targets can effectively reduce hospitalization and surgery rates in IBD patients, providing good effectiveness and high safety.¹³⁹ Currently, biologics for the clinical treatment of IBD mainly use the following three strategies to achieve targeted immunosuppression: 1. Neutralization of pro-inflammatory cytokines (anti-TNF antibodies) 2. Regulation of immune cell subset differentiation and function (anti-IL-12/IL-23 antibodies) 3. Inhibition of

lymphocyte recruitment and localization (anti- $\alpha 4\beta 7$ integrin antibodies).¹⁴⁰⁻¹⁴² (Figure 5) Moreover, the development of novel small molecule drugs offers a new option for IBD treatment. They offer advantages such as non-immunogenicity, high oral bioavailability, short half-life, and low production costs.¹⁴³ The new small molecule drugs approved by the FDA for IBD treatment include JAK inhibitors and S1PR modulators.¹⁴⁰⁻¹⁴² (Figure 5) As conserved mediators of cytokine and growth factor signaling, JAKs interact with STAT to mediate downstream biological effects, such as inducing the expression of pro-inflammatory cytokines.¹⁴⁴ S1P/S1PR1 interactions are essential for the trafficking of naïve and activated T and B cells. Continuous exposure to effective concentrations of S1PR agonists leads to persistent receptor downregulation and lymphocyte sequestration in lymphoid tissues, which may prevent them from reaching the site of inflammation.⁹⁵

Despite the promising potential of pharmacotherapy for IBD, there remain significant challenges, including drug resistance, off-target systemic side effects, severe immunosuppression, and concerns about long-term safety. In clinical practice, a variety of strategies have been explored to enhance treatment outcomes for IBD, including monitoring drug levels, adjusting the sequence of drug administration (either escalating or de-escalating), optimizing drug delivery systems, and employing combination drug therapies. However, the long-term remission rates and safety continue to be unsatisfactory.^{145,146} Further optimizing drug regimens and developing new treatment strategies are essential for improving the clinical treatment of IBD.

Microbial therapy

Since the causal relationship between intestinal microbiota imbalance and inflammatory response in IBD patients remains unclear, their complex interaction may be a significant factor limiting the effectiveness of immunosuppressive therapy. For IBD patients with severe abnormal intestinal microbiota, merely regulating the immune response cannot effectively improve the intestinal microecology, leading to ineffective treatment or recurrence after remission. Therefore, therapeutic interventions that directly target intestinal microecology seem to be an important direction for development. Currently, microbial therapies widely studied include FMT, phage therapy, and microecological modulators, such as probiotics, prebiotics, and synbiotics. They are

apical junction complex and its associated actin regulatory proteins, such as myosin IIA and ezrin.¹²⁴ However, AhR ligand levels are significantly reduced in IBD patients, and microbiota involved in tryptophan metabolism, such as *P.russellii*, *Lactobacillus*, and *Bifidobacteria* are also decreased.¹²⁵ Administering a *Lactobacillus* strain with high tryptophan-metabolizing capabilities could improve damaged microbiota-derived AhR ligand signaling in the host, thereby ameliorating intestinal inflammation.¹²⁶

Short-chain fatty acids. Intestinal microbiota ferments dietary carbohydrates into SCFAs, including acetate, propionate, and butyrate. These are crucial energy sources for colon cells and play a vital role in maintaining intestinal homeostasis.¹²⁷ SCFAs, particularly butyrate, have anti-inflammatory effects, including inhibiting LPS-induced NF- κ B activation and pro-inflammatory cytokine expression and promoting AMPs and anti-inflammatory cytokine expression. They stimulate Bregs and Tregs proliferation and function by signaling through G-protein-coupled receptor (GPCR) pathways or inhibiting histone deacetylases (HDACs) in IECs and immune cells, such as macrophages, Neutrophils, DCs, and B cells.¹²⁸⁻¹³¹ SCFAs also inhibit the progression of IBD by inhibiting excessive TLR signaling and regulating the assembly and disassembly of NLRP3 inflammasomes.¹³² Additionally, SCFAs enhance the expression of TJPs in the colon, thereby maintaining intestinal barrier integrity by strengthening the junctions between IECs and promoting the polarization of intestinal cells.¹³³ SCFAs produced by the microbiota can also directly inhibit pathogens. For example, exposing *S. enterica* to butyrate downregulates the expression of virulence genes and reduces its capacity to invade host cells or trigger apoptosis.¹³⁴ Studies have indicated a significant decrease in the abundance of SCFA-producing bacteria in the intestinal mucosa and feces of IBD patients. This decrease is closely associated with a reduced SCFA content in the feces of these patients.^{135,136} The population of *A. muciniphila*, one of the SCFAs-producing bacteria, and SCFAs concentration have been found to decrease in DSS-treated mice, while FMT enriched with *A. muciniphila* and SCFAs improved DSS-induced colitis in mice.¹³⁷

CLINICAL TREATMENT OF IBD

Immunosuppressive therapy

As an autoimmune disease influenced by complex factors, including

Table 3. Clinical trials supporting the efficacy of probiotics, prebiotics, and synbiotics

Interventions	Duration	Conditions	Conclusion	References	
Probiotics	<i>B. longum</i> 536	8 weeks	Mild-to-moderately active UC	Relieves clinical symptoms and reduces endoscopic index and the Mayo subscore.	271
	VSL#3 ^a	12 weeks	Mild-to-moderately active UC	Achieves clinical responses and remissions safely and effectively.	272
	VSL#3 ^a	1 year	Active UC in children	Induces and maintains UC remission and without adverse events.	273
	<i>L. rhamnosus</i> , <i>L. plantarum</i> , <i>L. acidophilus</i> , and <i>Enterococcus faecium</i>	4 weeks	Asymptomatic IBD	Relieves intestinal inflammation in UC patients and is well tolerated.	274
	<i>Lactobacillus</i> and <i>Bifidobacterium</i> species (Probiotic 10 billion active cells [®])	6 weeks	Mild-to-moderately active UC	Induces remission in UC patients significantly.	275
	Hyperbiotics Pro-15 ^b	3 months	IBD	Reduces oxidative stress values and improves clinical symptoms.	276
Prebiotics	FOS-enriched inulin	2 weeks	Active UC	Reduces calprotectin in feces and is well tolerated.	156
	Oral microencapsulated sodium butyrate	12 months	UC in clinical remission	Maintain remission and improves residual symptoms.	277
	FOS	3 weeks	Active ileocolonic CD	Increases fecal <i>bifidobacteria</i> concentrations and modifies mucosal dendritic cell function.	278
Synbiotics	Probiotics: <i>E. faecium</i> , <i>L. plantarum</i> , <i>S.thermophilus</i> , <i>B. lactis</i> , <i>L. acidophilus</i> , and <i>B. longum</i> Prebiotics: FOS	8 weeks	Mild-to-moderately active UC	Improves clinical activity significantly and appears to positively affect the acute phase reactants and endoscopic activity levels.	279
	Probiotics: <i>B. longum</i> Prebiotics: FOS/inulin mix	4 weeks	Active UC	Improves the full clinical appearance of chronic inflammation.	280
	Probiotics: <i>L. casei</i> , <i>L. acidophilus</i> , <i>L. rhamnosus</i> , <i>L. bulgaricus</i> , <i>B. breve</i> , <i>B. longum</i> , and <i>S. thermophiles</i> Prebiotic: FOS (Lactocare [®])	8 weeks	Mild-to-moderately active UC	Relieves clinical symptoms	281

^aVSL#3: *L. acidophilus*, *L. plantarum*, *L. casei*, *L. delbrueckii* subspecies *bulgaricus*, *B. breve*, *B. longum*, *B. infantis*, and *S. salivarius* subspecies *thermophiles*.

^bHyperbiotics Pro-15: *L. plantarum*, *L. fermentum*, *L. acidophilus*, *B. infantis*, *L. casei*, *B. longum*, *L. rhamnosus*, *B. lactis*, *L. reuteri*, *L. salivarius*, *L. paracasei*, *L. gasseri*, *B. bifidum*, *B. breve*, and *S. thermophiles*.

Abbreviations: CD, Crohn's disease; FOS, fructooligosaccharides; IBD, Inflammatory bowel disease; UC, ulcerative colitis.

expected to become safer and more sustainable treatments.

Microecological modulators. Probiotics are live microorganisms that provide numerous health benefits, including inhibiting pathogens, regulating the immune system, and enhancing the intestinal barrier.¹² Commonly used probiotics include *Bifidobacterium*, *Lactobacillus*, *E. coli* Nissle 1917 (EcN), *S. boulardii*, and *Enterococcus*.¹⁴⁷ The guidelines from the European Crohn's and Colitis Organization state that EcN is an effective alternative to mesalazine for maintaining remission in UC patients.¹⁴⁸ Studies have shown that mixed strains produce more significant effects than single strains.¹⁴⁹ Several types of probiotic cocktails have been used in clinical trials, among which VSL#3 has been the most extensively studied. VSL#3 is a lyophilized mixture of four *Lactobacillus* strains (*L. casei*, *L. plantarum*, *L. acidophilus*, and *L. delbrueckii* subsp. *Bulgaricus*), three *Bifidobacterium* strains (*B. longum*, *B. breve*, and *B. infantis*) and *S.salivarius*.¹⁵⁰ It can protect the intestinal barrier by improving the intestinal microbiota, promoting mucus production, enhancing TJs, and inhibiting pro-inflammatory signal transduction.¹⁵⁰ Moreover, probiotic supplementation can potentially enhance drug efficacy. For example, the combination of *S. boulardii* and mesalazine can mitigate IBD symptoms and reduce adverse effects.¹⁵¹

Prebiotics are substrates selectively fermented by the intestinal microflora, providing health benefits to the host. They mainly consist of fructooligosaccharides (FOS), inulin, galactooligosaccharides (GOS), and lactulose.¹⁵² FOS can enhance TJ assembly in IECs through the AMPK-dependent pathway.¹⁵³ Supplementing with FOS can mitigate the DSS-induced loss of mucosal mucin and epithelial TJPs, and reduce the expression of pro-inflammatory cytokines, thus improving the IEB and regulating the immune response.¹⁵⁴

Inulin is a mixture containing various fructans with different degrees of polymerization. Studies have shown that the clinical symptoms of CD patients significantly improve after consuming FOS-enriched inulin, a change that positively correlates with the increased count of *B. longum* in feces.¹⁵⁵ In active UC, dietary supplementation with FOS-enriched inulin is well tolerated and associated with early normalization of fecal calprotectin.¹⁵⁶ Administering GOS orally to DSS-induced colitis mice can significantly alleviate symptoms of colitis, such as weight loss and colon shortening, and decrease the expression of inflammatory mediators such as IL-6 and TNF- α .¹⁵⁷

When probiotics and prebiotics are combined in products to exert synergistic effects, they are often referred to as synbiotics. They are more effective than prebiotics or probiotics alone and reduce cost and treatment duration, thus improving compliance.¹⁵⁸ Synbiotics enhance the beneficial properties of their two components by improving the survival and metabolism of specific bacterial strains. Therefore, choosing the right substrate and microbial combination for the synbiotic effect is crucial.¹⁵⁹

Currently, numerous clinical trials have demonstrated that probiotics, prebiotics, and synbiotics can induce and maintain remission in IBD. (Table 3) However, the limited sample size, potential bias, and substantial heterogeneity in current studies have led to a lack of acknowledgment by several guidelines of microbiome modulators as a recommended treatment option for IBD.¹⁴⁰ Furthermore, due to variations in patients' dietary habits, disease progression, and intestinal microbiota, certain studies may have unsatisfactory therapeutic outcomes when using microbial modulators. This could be due to inadequate intervention components, inappropriate therapeutic doses, or insufficient treatment duration.¹² In the future, research and application of

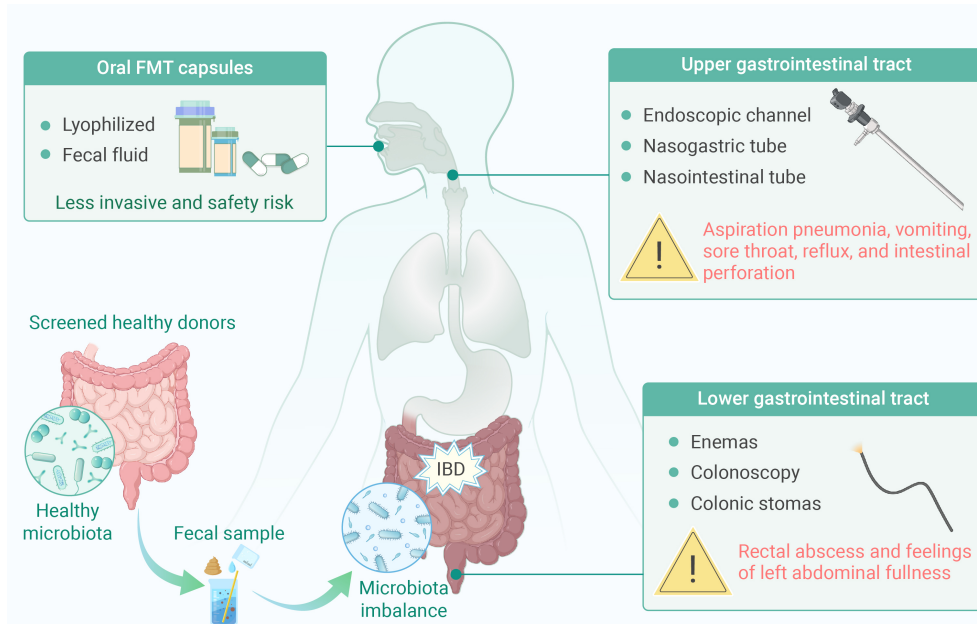


Figure 6. Process and adverse events of FMT for IBD.

scopic enteral tubing (TET) enabled multiple FMTs and targeted intestinal administration through an indwelling tube, improving operational safety and patient satisfaction.¹⁷⁰⁻¹⁷² Oral FMT is less invasive and carries a relatively low safety risk, making it a promising emerging delivery option.^{173,174}

In addition to considering potential adverse reactions, the therapeutic effect is crucial in selecting the route of administration. Due to the heterogeneity in study designs for FMT, evaluating the effect of the delivery route on efficacy is challenging. However, preliminary selection can be made based on the type and location of IBD. For example, enema or colonoscopy ensures maximum benefit in UC patients, while gastrojejunal administration is more effective in reducing intestinal lesions in cases of CD with ileal inflammation.¹⁷⁵ It has been suggested that

administering FMT via both upper and lower GIT routes may enhance the engraftment rate of donor strains.¹⁷⁶

Additionally, implementing complementary strategies can enhance the effectiveness of FMT. For example, FMT combined with an anti-inflammatory diet, followed by continuing with an anti-inflammatory diet alone, effectively induces and maintains UC remission.¹⁷⁷ Optimizing bowel preparation before FMT, including pretreatment with antibiotics, can enhance the effectiveness of FMT treatment by facilitating microbiota colonization.¹⁷⁸ However, antibiotics assist in the colonization of donor flora by reducing the biotic resistance in the recipient's intestinal microbiota. It is crucial to consider the dosage, type, and timing of antibiotics to prevent exacerbating intestinal microbiota imbalance.¹⁷⁹

Phage therapy. Phages are viruses that infect and destroy their host bacteria through processes like replication-lysis or the lysogenic cycle. The application of exogenous phages to regulate the structure of intestinal microbiota is a novel therapeutic strategy for IBD. Currently, phage therapy for IBD mainly targets AIEC and *Klebsiella pneumoniae*. When a mixture of three AIEC-targeted phages was administered for 2 weeks for antimicrobial therapy in a DSS-induced colitis model in AIEC-colonized mice, a significant reduction in AIEC abundance was observed in fecal and intestinal samples, leading to the amelioration of colitis symptoms.¹⁸⁰ In a double-blinded, placebo-controlled crossover trial of *E. coli*-targeting phages, subjects with the cocktail phages for 28 days revealed a selective reduction in fecal *E. coli* load without disrupting the global microbiota.¹⁸¹ Oral phage mixture against the IBD-associated bacterial strain *K. pneumoniae* reduced colonization of the target bacteria and immune cell infiltration and alleviated colitis in Kp-2H7 mono-colonized mice.¹⁸² However, phage-mediated therapeutic outcomes are not always effective. Studies have shown that oral phage delivery did not observe significant phage replication, a decrease in the total *E. coli* count and an improvement in intestinal condition.¹⁸³ While the treatment with phage is emerging as an IBD therapeutic strategy, the quantity and quality of phage research need to be improved to provide more reliable perspectives.

It is worth noting that not all phages are suitable for IBD treatment. The inherent ability of lysogenic phages to mediate gene transfer between bacteria may increase virulence or resistance to effective treatment.¹⁸⁴ However, the removal of lysogenic genes in the genome through emerging synthetic biology techniques is expected to give them clinical therapeutic value. This provides a potential way to kill bacteria such as *C. difficile* that have not yet been found to be targeted by toxic phages.¹⁸⁴ Another crucial aspect of utilizing phages for treatment of IBD is ensuring that they evade recognition by the human immune system to prevent triggering an adverse immune reaction. There were studies showing that *Lactobacillus*, *Escherichia*, and *Bacteroides* phages could be recognized by TLR9 resulting in the production of INF- γ .¹⁸⁵

microbial ecology treatments should adhere to the principle of individualized medication. We need to further elucidate the specific mechanisms through which the microbiota and various nutrients regulate intestinal microecology.

Fecal microbiota transplantation. As an emerging microbial therapy, FMT aims to reshape the microecology by transferring screened and treated donor fecal samples into the recipient's intestine. (Figure 6) It has been used to treat *C. difficile* infection, but it is still in the clinical trial stage for IBD.¹⁶⁰ A consensus is already emerging that FMT can effectively induce remission for mild to moderate UC. However, there is still insufficient evidence to support its use as a routine clinical treatment option.^{10,160} In 2015, the first randomized controlled trial (RCT) assessing the effectiveness of FMT for active UC demonstrated that the remission rate in the FMT group was significantly higher than in the placebo group, with no apparent difference in adverse events.¹⁶¹ A recent meta-analysis of high-quality RCTs on FMT for UC also showed that the clinical and endoscopic remission rates in the FMT group (50.17% and 82.39%) are better than those in the control group (29.02% and 60.10%). There was no significant difference in the incidence of adverse events between the two groups ($P = 0.96$).¹⁶² However, UC is a chronic disease with a high recurrence rate, and the absence of long-term follow-up data limits the current assessment of FMT for maintaining remission. A small sample study conducted over 24 months suggests that a single dose of FMT induces long-term remission in patients with active UC and effectively reconstructs the composition of intestinal microbiota.¹⁶³ However, the results of another study with a 12-month follow-up do not support the use of a single-dose FMT to maintain remission in UC. Based on previous studies, the researchers believed that repeated FMT treatments were necessary to maintain remission.¹⁶⁴ Because the lesion sites and phenotypes of CD are more heterogeneous than those of UC, they present more challenges to the determination of FMT effectiveness. Preliminary studies suggest that FMT could be a successful treatment for CD. Further RCTs are needed to provide more evidence.¹⁶⁵⁻¹⁶⁷

There is no significant difference in disease deterioration or adverse events between the FMT and control groups, with a low incidence observed. This finding suggests that the safety concerns associated with FMT are not due to the donor feces but may be related to the method of administration.¹⁰ (Figure 6) Currently, fecal material is administered through the upper gastrointestinal tract (GIT), the lower GIT, or orally. Upper GIT routes mainly include endoscopic channel, nasogastric tube, and nasointestinal tube. These may cause adverse symptoms such as intestinal perforation, aspiration pneumonia, vomiting, sore throat, and reflux.^{168,169} Lower GIT routes mainly include enemas, colonoscopy, and infusions through colonic stomas. These may lead to rectal abscess or mental stress in patients caused by repeated colonoscopy examinations.^{168,169} In 2016, the development of transendo-

Moreover, phage-mediated bacterial killing may lead to excessive accumulation of proteins, lipopolysaccharides, and nucleic acids, thereby exacerbating inflammation.¹⁸⁶ The balance between these intestinal adverse effects and the improvement of bacterial structure in IBD treatment requires further investigation.

Current challenges and future directions

Although many clinical trials have shown that microbial therapies can induce and maintain remission in IBD, their ability to fully demonstrate effectiveness is limited. First, variations in methodology among studies limit the interpretation of the overall findings. The studies vary in the type and dosage of prebiotics and probiotics, the preparation process, delivery method, and frequency of FMT, as well as the administration route, dose and duration of phage therapy. The differences in disease subtypes, disease progression, and patients' previous treatment history also directly affect treatment effectiveness. Additionally, most studies have not considered the impact of dietary habits on the effectiveness of microbial therapies, despite their role as a risk factor for IBD and their involvement in the regulation of intestinal microecology.¹⁸⁷ Due to a lack of thorough understanding of how the microbiota maintains intestinal homeostasis and triggers inflammatory responses, the differences introduced by the study design cannot be eliminated through analysis. In addition to methodological diversity, the small sample sizes and insufficient follow-up duration in the studies limit the reliability of the findings. It is crucial to carry out large-scale, long-term follow-up RCTs with controlled variables to validate the effectiveness and optimize treatment regimens for microbial therapies that have shown positive outcomes.

Moreover, numerous challenges persist in transitioning microbial therapies from clinical trials to routine practice. The intestinal microbiota has heterogeneity in terms of species and quantity both among individuals and within the intestine itself. Differences in disease severity and the affected sites in IBD also suggest that changes in the microbiota have individual-specific characteristics.¹⁴ Therefore, microbial therapy needs to be tailored for individualized and precise treatment. It requires understanding how different microbes support, tolerate, or reject each other in intestinal microecology and the specific mechanisms through which they interact with their hosts. According to the changes in the intestinal microbiota observed in IBD patients, the use of individualized microecological modulators that can function cooperatively, persist within the intestine, and enhance disease resistance will improve the therapeutic outcome.¹⁸⁸ For FMT, selecting the right donor is also critical to achieving precise treatment.¹⁸⁹ The existing donor screening criteria for FMT in IBD indications only rely on exclusionary approaches, and there are no known characteristics to identify the ideal donor.¹⁶⁸ It has been reported that the microbial diversity of donor feces is related to the effectiveness of FMT.¹⁹⁰ Multidonor FMT has the potential to enhance therapeutic effects by increasing species diversity.¹⁹¹ However, the high diversity of the transplant microbiota is not the only factor affecting effectiveness; the high abundance of specific *Bacteroides* species (*B. fragilis* and *B. finegoldii*) in mixed donor feces suggests better FMT treatment outcomes.¹⁹² Additionally, the clinical application of microbial therapies requires identifying biomarkers that can assess effectiveness and safety. Studies have shown that an increase in butyrate-producing bacteria such as *Clostridium* clusters IV and XIVa, as well as an overall increase in butyrate production capacity in UC patients who undergo FMT, predicts a sustained response. Conversely, a relapse is associated with the enrichment of *Bacteroidetes* and *Proteobacteria*.¹⁹³ Further research is needed to validate the accuracy of potential biomarkers and discover new predictors to advance the development of microbial therapies.

As an emerging IBD treatment strategy, phage therapy still has many controversies and unknowns to be further studied. Although phage therapy does not typically lead to serious life-threatening adverse reactions, findings suggest that oral administration of *E. coli* phages can result in transient abdominal pain, dyspepsia, and toothache.^{194,195} These symptoms may be caused by bacterial lysis of phages, direct interactions between phages and the mammalian host immune system, or residual bacterial toxins in phage products. The safety of phage therapy still needs to be considered. Furthermore, resistance to phage therapy seems inevitable as bacteria can quickly evade phage attacks through a series of antiviral mechanisms, eventually

resulting in the uncontrolled proliferation of phage-resistant mutants and treatment failure. Most current studies predominantly examine the efficacy and safety of phage therapy, with limited attention given to the investigation of phage resistance. In 12 relevant clinical studies, 7 cases confirmed the emergence of phage resistance during treatment.¹⁹⁶ There have been reported feasibilities of oral combined phage therapy in avoiding drug resistance.¹⁹⁷ The development of effective phage combinations requires further study of the impact of phages on the intestinal microbiota structure and the targets of emerging drug-resistant bacteria. Moreover, to maintain the in vivo therapeutic efficacy of phage, it is crucial to improve the stability and activity of phage combinations by optimizing the phage encapsulation strategy and delivery pathway.

FRONTIER PRECLINICAL MODEL: ORGANOID

To provide a solid theoretical foundation for microbial therapy, optimizing preclinical research on intestinal microecology is crucial. Due to the technical and ethical constraints in collecting human intestinal microbiota and the challenges in eliminating interfering variables, traditional studies usually rely on mouse models. They can control diet, environment, genetics, and microbiota structure and facilitate sample collection.¹⁹⁸ However, the species-specific intestinal anatomy and microecology of mice cannot fully represent the characteristics of the human intestine, limiting the transformation of preclinical research results to clinical applications.^{199,200} The successful construction of intestinal organoids in 2009 indicates a new era of intestinal flora and IBD research.²⁰¹

Development of organoid models

Intestinal organoids are mainly derived from adult human intestinal stem cells and induced pluripotent stem cells.^{201,202} Under the embedding of Matrigel, the extracted cells proliferate, differentiate, and self-assemble into a miniaturized three-dimensional (3D) structure that resembles the intestine *in vitro*. They maintain the cell lineage, organizational characteristics, and certain functions of the source region.^{203,204} Organoids from uninflamed intestinal tissue of IBD patients can preserve patient-specific transcriptomic signatures.²⁰⁵ However, some studies have indicated that organoids from inflamed regions lose the acute inflammatory phenotype during the culture process and require stimulation with pro-inflammatory factors for re-induction.^{206,207} Since 3D organoids are closed structures that mimic the intestinal lumen, their applications in investigating intestinal microecology require appropriate techniques to facilitate the interaction of the microbiota with the epithelial apex located on the inner surface of the lumen. The technologies currently applied mainly include microinjection, polarity reversal, and linearization.²⁰⁸

Microinjection involves directly introducing microbiota into the differentiated lumen, and its significant advantage is to retain the 3D structure of the organoids.²⁰⁹ The closed organoid lumen has hypoxia characteristics, making it favorable for the growth of facultative or obligate anaerobic bacteria, although it not be suitable for investigating specific aerobic bacteria.²¹⁰ To address the issues of low bacterial throughput and the risk of leakage toward the basal side in manual bacterial injection, researchers are continuously optimizing this technique. Employing fluorescent bacteria for visualizing and evaluating injections, performing repeated microinjections, and establishing a high-throughput microinjection platform are possible solutions.^{208,210}

3D organoid polarity reversal can turn the epithelial apical surfaces outside. The technique is to use the Calcium-chelator EDTA to destroy the ECM protein laminin, followed by cultivation on a low-binding plate to achieve reverse polarization. Subsequently, the microbiota can be directly added to the medium to contact with the apical side of the epithelium, which can increase the experimental throughput.²¹¹ However, there are still problems to be solved in this technique, such as incomplete polarity reversal, adhesion due to ECM defect, difficulty in medium change, and inapplicability of anaerobic bacteria.²⁰⁸ Furthermore, although the reverse polarized organoids demonstrate appropriate epithelial barrier integrity and nutrient absorption, it is necessary to conduct further verification to determine whether this polar reversal impacts the interaction between microbiota and epithelium.

Linearization of organoids is to fragment 3D organoids and seed them onto collagen hydrogel or transwell membrane treated with ECM to form a mono-

layer organoid.^{212,213} They retain the characteristics of the intestinal epithelium, such as barrier function, gene expression, and polarization.^{212,213} Similar to reverse polarized organoids, the microbiota can be directly added to the medium for co-culture, supporting high-throughput experimental design. Nevertheless, a significant limitation of linearized organoids is the loss of essential spatial structure. In addition, the system also faces challenges in introducing anaerobic bacteria into the aerobic environment of organoid culture. To overcome this limitation, researchers have improved the linearized organoids model. The enteroid-anaerobe co-culture system and the intestinal hemi-anaerobic co-culture system can achieve a dynamic oxygen gradient from the apical to the basolateral side of the single-layer IEC.^{214,215} Both systems supply oxygen only to the basolateral side, which does not reach the top after being consumed by the IEC, leaving the apical region hypoxic and conducive to the introduction of anaerobic bacteria.^{214,215}

The models developed through the aforementioned techniques can facilitate the co-culture of microbiota and organoids. However, there remain significant differences between them and the human intestinal environment. The introduction of microfluidic chip technology provides better experimental control of integrated organoids through multiple connected microfluidic channels.²¹⁶ Organoid chips can independently circulate media through the epithelial apical and basolateral sides of linearized organoids, which facilitates medium replacement and prevents bacterial overgrowth and toxic accumulation, thereby prolonging the co-culture period.²¹⁷ Another advantage of microfluidics technology is that it can simulate fluid flow and peristaltic deformations. These mechanistic signals favor crypt-villi formation and epithelial multilineage differentiation.^{218,219} The differentiated goblet cells can secrete MUC2 to promote the formation of double-layer mucus. Compared with other organoid models, the mucus layer produced on the microfluidic chip closely resembles the human intestinal mucus structure.²²⁰ Some early-developed organoid chips have been marketed but are expensive and low-throughput. More optimized organoid chips need further development and application.

Currently, several models used to study the interaction between the host and microbiota in IBD have their advantages and disadvantages and are not optimal *in vitro* models. Balancing similarity to the human intestine, long-term co-culturing, high throughput, low cost, and simplicity of operation is the direction for improving future organoid models. In addition, the standardization of culture conditions and methods or the development of automation systems are key to applying organoid models in the laboratory.

Application of organoids in IBD

Based on the above techniques, organoid models have been used to investigate the impact of microecology dysbiosis on IBD progression. Compared to conventional cell lines, *C. difficile*-induced enteritis models based on jejenum-derived organoids exhibit lower susceptibility to bacterial toxins, despite having higher levels of toxin receptors. Organoids may delay toxin-epithelial interaction by secreting mucus that closely resembles the human intestinal environment.²²¹ It has been shown that colonization of EHEC in colon monolayer organoids can reduce mucus production and destroy the integrity of the colonic mucus layer.²²² Organoids can also be used to identify bacterial strains that can alleviate IBD. A study utilizing 3D organoids derived from patients with CD and UC found that cell-free culture supernatants originating from *Firmicutes* can attenuate IL-1 β -stimulated IL-8 production and NF- κ B signaling pathways in a strain-specific manner.²²³ In the co-culture system comprising organoids and lamina propria lymphocytes, *Lactobacillus* enhances the repair of intestinal organoid damage by inducing IL-22 secretion from lymphocytes.²²⁴ It is worth noting that the degree of diversity of intestinal microbiota in organoid-derived mice impacts research outcomes.²²⁵ Therefore when studying specific microbiota in organoids, the overall distribution as well as the microecology similarity to patients should be considered. Intestinal microbiota-related metabolites have also been extensively studied in organoid models. SBAs promote IEC renewal and drive regeneration in response to injury by activating TGR5 on organoids from mice.²²⁶ A study using 2D organoids derived from UC patients found that high acetate could reduce the expression of pro-inflammatory cytokines and protect the expression of epithelial resistance barrier genes.²²⁷ In 3D organoids sourced from iPSCs of a pediatric CD patient, butyrate reduces toxin-induced epithe-

lial ROS production and ECM gene expression implicated in intestinal strictures, thus suppressing potential stenosis formation.²²⁸

Intestinal organoids were also applied to investigate the role of inflammation and IEB damage in the pathogenesis of IBD. The levels of inflammatory cytokines in colonic organoid cultures are lower than those in the original mucosal specimens, and their overexpression requires restimulation.²⁰⁶ Introducing a cytokine mixture containing TNF- α , IFN- γ , and IL-1 β to the intestinal organoids sourced from CD patients can mimic the inflammatory microenvironment. These cytokines affect the expression of cell junction proteins and significantly destroy the epithelial barrier of intestinal organoids.²²⁹ TNF- α , a pro-inflammatory cytokine highly expressed in IBD, leads to a rapid loss of organoid integrity and the emergence of morphologically altered cells.²³⁰ Inhibitors of apoptosis, crucial in regulating TNF-mediated survival, death, and inflammatory signaling pathways, show promise in alleviating organoid damage and have the potential for treatment of IBD.^{230,231} Moreover, in IBD-derived intestinal organoids, TNF- α reduces the response to butyrate by decreasing the uptake, oxidation, and depletion of the intestinal epithelium, disrupting the beneficial effects of butyrate supplementation during active inflammation.²³²

Intestinal organoids also provide a convenient platform for investigating the genetic risk factors for IBD, preserving region-specific gene expression patterns and epigenetic characteristics of the original tissue.^{233,234} A study comparing intestinal organoids sourced from pediatric UC and CD patients has found differences in DNA methylation and transcriptional patterns in the colon epithelium, which implies a role of differential diagnosis and gene therapy for IBD.²³⁵ However, since the current organoid culture cannot fully mimic the *in vivo* environment, long-term culture may lead to changes in global DNA methylation levels.²³⁶ Organoids derived from tissues exhibiting varying inflammatory states differ in the rate of methylation changes.²³⁷ So when analyzing data from organoids, the number of passages should be considered as a potential confounding factor, particularly when the organoids are at late-passage. Not only by gene profiling of the tissue-derived organoids, genetic manipulation of the IBD-associated risk genes within organoid cultures using transposon mutagenesis, siRNAs, and CRISPR-Cas techniques enhances our understanding of IBD genes and the epithelial cell function.²³⁸ Combining organoids with molecular biology techniques is a crucial tool for IBD research.

In addition to the mechanism study for IBD, organoids are employed in exploring potential therapeutic strategies. Observation of the inflammatory state, barrier function or gene expression of intestinal organoids after drug intervention provides useful insight to evaluate the efficacy of new drugs *in vitro*.²³⁹ In organoid models, the success rate of clinical translation of IBD treatments has been significantly improved.²⁴⁰ Intestinal organoids can also be directly used in IBD therapy. In 2012, a research team first implanted organoids derived from a single Lgr5⁺ stem cell into DSS-induced colitis mice by enema and found that it can repair damaged epithelial cells. Transplanted organoids accurately target and colonize superficially damaged tissues, reconstructing the monolayer epithelium and repairing crypt structures.²⁴¹ They provided a detailed protocol to facilitate the implementation of this transplant model in 2022.²⁴² Subsequently, the researchers announced that they had successfully transplanted organoids into the intestinal mucosa of patients with refractory UC, but specific clinical data have not been published. As a new therapeutic strategy, the safety of organoid transplantation in the clinical application remains further investigated, including immune rejection and the risk of tumors. Furthermore, the precise delivery and sufficient stay of the organoids at the target tissue is essential to repair the damaged mucosa.

PROSPECT

The relationship between intestinal microecology dysbiosis and IBD progression is complex. Understanding its physiological structure, function, and the specific mechanisms that trigger the host's abnormal inflammatory response under pathological conditions will provide insights into the prevention, diagnosis, and treatment of IBD. Currently, numerous clinical trials are underway regarding microbial therapies for IBD, and the existing results show promising prospects for their application. However, limitations in assessing the efficacy of microbial therapy persist due to our incomplete understanding of the intestinal microbiota and variations in study design. Future efforts

should focus on studying the mechanisms of interaction between the intestinal microbiota and the host by using frontier preclinical models such as organoids to provide a robust theoretical foundation for clinical diagnosis and treatment. Moreover, there is a pressing need for higher-quality clinical trials to validate the efficacy and optimize the treatment strategies for existing effective microbial therapies. It is worth noting that personalized precision medicine is essential for clinical practice due to the variability of intestinal microbiota among individuals and within the intestine, as well as the varied disease progression and affected sites in IBD. Identifying the specific characteristics and pathogenic mechanisms of intestinal microecology in IBD patients will provide a more effective strategy for the clinical treatment of IBD.

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AUTHOR CONTRIBUTIONS

Kaichun Wu and Limin Xia offered directions on this manuscript. Yue Yin drafted the manuscript. Yue Yin, Weibo Feng, Xilang Chen, and Jie Chen made the figures and table. Yue Yin, Weibo Feng, Xilang Chen, Jie Chen, and Guodong Wang reviewed the literature. Kaichun Wu and Limin Xia reviewed the manuscript and gave comments. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

Not applicable.