

## Review article



# Microbiota in inflammatory bowel disease: mechanisms of disease and therapeutic opportunities

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## Abstract

Perturbations in the intestinal microbiome are strongly linked to the pathogenesis of inflammatory bowel disease (IBD). Bacteria, fungi and viruses all make up part of a complex multi-kingdom community colonizing the gastrointestinal tract, often referred to as the gut microbiome. They can exert various effects on the host that can contribute to an inflammatory state. Advances in screening, multiomics and experimental approaches have revealed insights into host–microbiota interactions in IBD and have identified numerous mechanisms through which the microbiota and its metabolites can exert a major influence on the gastrointestinal tract. Looking into the future, the microbiome and microbiota-associated processes will be likely to provide unparalleled opportunities for novel diagnostic, therapeutic and diet-inspired solutions for the management of IBD through harnessing rationally designed microbial communities, powerful bacterial and fungal metabolites, individually or in combination, to foster intestinal health. In this Review, we examine the current understanding of the cross-kingdom gut microbiome in IBD, focusing on bacterial and fungal components and metabolites. We examine therapeutic and diagnostic opportunities, the microbial metabolism, immunity, neuroimmunology and microbiome-inspired interventions to link mechanisms of disease and identify novel research and therapeutic opportunities for IBD.

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## Introduction

Inflammatory bowel disease (IBD) is a heterogeneous group of inflammatory diseases affecting an estimated 0.3–0.5% of the global population<sup>1</sup>. The condition is broadly divided into Crohn's disease (CD) and ulcerative colitis (UC), which involves inflammation of the gastrointestinal tract or the colon, respectively<sup>2</sup>. The aetiology of IBD is complex and multifactorial, involving genetic, environmental, dietary, immune and neuronal factors, all of which are interlinked by a complex microbial community (microbiome) of bacteria, fungi, archaea, viruses and protozoa. The microbiome plays a central role in IBD, as evidenced by genetic and serological markers and alterations in pathways responsible for host–microbiota interactions, microbial alterations in first degree relatives and by microbial change associated with resolution of inflammation. Indeed, the rising incidence of IBD globally parallels change in environmental factors (Box 1) that often affect the microbiome<sup>3</sup>. The composition and distribution of microbial species and strains with unique or redundant function varies from individual to individual, adding another level of complexity. Recent advances in multiomics approaches, including metagenomics, metatranscriptomics and metabolomics, as well as fine-tuned experimental approaches and CRISPR-based genomics in mouse models, human, fungal and bacterial cells, have deepened our understanding of the role of the microbiota in IBD and are discussed in this Review.

## Microbiota composition and functional characteristics

The microbiota co-exists in a symbiotic state with the host, contributing to physiological processes, including digestive and metabolic

function, regulation of immune responses, protection against pathogen colonization and neuronal stimulation. When this balance is disrupted, specific microbes and their metabolites can affect inflammation<sup>4–6</sup>. Furthermore, microbiota modalities, structured as a multibacterial biomarker panel, have shown promise as a noninvasive tool for IBD diagnosis, highlighting diagnostic applicability<sup>7</sup>.

## Bacteria

Fluctuations in the composition and diversity of gut bacteria, which constitute the vast majority (over 99%) of the gut microbiome<sup>8</sup>, have been associated with IBD<sup>9,10</sup>. The most dominant bacterial groups in the gut are Firmicutes and Bacteroides, which together constitute approximately 90% of gut microbiota<sup>11</sup>, and are key producers of short-chain fatty acids (SCFAs)<sup>12</sup>. Although bacterial 'dysbiosis' in IBD has been typically characterized by an increased ratio of potentially pathogenic to beneficial bacteria and lower overall diversity<sup>13,14</sup>, this paradigm is now shifting. The focus of microbiome research is moving from microbial composition to the functional characteristics of specific bacterial species and strains, along with the integration of multiomics datasets to better infer function. Such comprehensive multiomics investigations have elucidated temporal alterations in the composition of specific bacterial species and their metabolic profiles in patients with IBD, more accurately pinpointing alterations in bacterial ecology that coincide with disease initiation and progression<sup>10,15,16</sup>.

With respect to specific bacterial species, several large-scale cohort studies have demonstrated overrepresentation of certain bacterial groups and species in active IBD, including Enterobacteriaceae, *Fusobacterium* spp., *Ruminococcus gnavus*, *Streptococcus anginosus*, *Enterococcus* spp., *Campylobacter* spp., Gammaproteobacteria and Deltaproteobacteria, and a decrease in beneficial groups such as *Faecalibacterium prausnitzii*, *Christensenellaceae*, *Collinsella* spp., *Roseburia* spp. and *Ruminococcus* spp.<sup>17–20</sup>. A shotgun metagenomic sequencing study found that levels of beneficial bacteria such as *Bifidobacterium longum* (in patients with UC), and *Eubacterium rectale* and *F. prausnitzii* (in both CD and UC<sup>19,21,22</sup>), were reduced compared with healthy individuals, whereas species such as *Bacteroides fragilis* were increased<sup>19</sup> (Table 1). *F. prausnitzii* is one of the most abundant species in the human gut<sup>23,24</sup> and is a key producer of butyrate, a SCFA with anti-inflammatory properties<sup>24</sup>. Several peptides with anti-inflammatory properties produced by *F. prausnitzii* have been identified, adding to the mechanisms through which it may exert its beneficial effects<sup>25</sup>. An early study exploring mucosa-associated microbiota of individuals with CD determined that *F. prausnitzii* reduction is associated with a higher risk of ileal CD recurrence<sup>20</sup>. Additional studies have shown a *F. prausnitzii* decrease in IBD<sup>19,21,22</sup>. The reduction of SCFA producers such as *F. prausnitzii* and *Roseburia hominis*<sup>10,15,16</sup>, coincides with an increase of adherent-invasive *Escherichia coli* in patients with IBD<sup>26–28</sup>. Increased transcriptional activity by *Clostridium* spp. (*Clostridium hathewayi* and *Clostridium bolteae*) and *R. gnavus* during IBD progression, indicated that these bacteria may be involved in disease pathogenesis<sup>15</sup>. *R. gnavus* and *Ruminococcus torques* glucorhamnan has been linked to inflammation in CD<sup>29</sup> and an antagonistic relationship with the potentially beneficial species *Akkermansia muciniphila*<sup>30,31</sup>. Other *Clostridium* spp. such as *Clostridium innocuum* and *Clostridium symbiosum* can even translocate into mesenteric adipose tissues of individuals with CD<sup>32</sup>. The gut microbiome composition of individuals with IBD would periodically resemble that of healthy individuals, following a dysbiotic deviation that precedes the disease relapse<sup>16</sup>. Furthermore, the overall balance and composition of the gut microbiota

## Box 1 | Food emulsifiers, herbicides, sugars and loss of hypoxia as factors in IBD

An integrated approach combining publicly available databases, chemical screens, machine learning and mouse models identified several environmental or food-derived factors associated with intestinal inflammation<sup>206</sup>. The herbicide propyzamide was found to promote inflammation in the large and small intestine through targeting of the aryl hydrocarbon receptor–NF-κB-C/EBPβ signalling axis in T cells and dendritic cells, and to reduce microbiome diversity in the ileum and the caecum<sup>206</sup>. Other environmental factors, including dietary and lifestyle changes associated with urban and industrialized lifestyles, can directly affect the microbiota and contribute to the pathophysiology of inflammatory bowel disease (IBD)<sup>13</sup>. Dietary components and habits are among the factors that profoundly affect IBD<sup>207,208</sup>. Carboxymethylcellulose emulsifiers disrupt the microbiome and mucosal barrier<sup>209</sup>, linking emulsifier and ultraprocessed food intake to IBD risk<sup>168</sup>. The loss of intestinal hypoxia during IBD can influence pathobiont overgrowth. In the inflamed intestine, *Escherichia coli*, a facultative anaerobe, uses reactive oxygen and nitrogen by-products for anaerobic respiration to grow and outcompete gut commensals<sup>210</sup>. *Candida albicans*, however, requires aerobic respiration to metabolize sugars, and its growth can be limited by probiotic *E. coli* consuming oxygen or by inducing epithelial hypoxia with the peroxisome proliferator-activated receptor-γ (PPARγ) agonist 5-aminosalicylic acid (also known as mesalamine)<sup>211</sup>, an anti-inflammatory agent used to treat mild-to-moderate ulcerative colitis.

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**Table 1 | Microorganisms and their functions associated with IBD**

Organism	Organism decreased or increased in IBD	Function	Function loss or gain
<b>Bacteria</b>			
<i>Faecalibacterium prausnitzii</i>	Decreased	SCFA production	Loss
<i>Roseburia intestinalis</i>	Decreased	SCFA production	Loss
<i>Eubacterium rectale</i>	Decreased	SCFA production	Loss
<i>Eubacterium hallii</i>	Decreased	SCFA production	Loss
<i>Sutterella</i> spp.	Decreased	SCFA production	Loss
<i>Gemmiger formicilis</i>	Decreased	SCFA production	Loss
<i>Ruminococcus bromii</i>	Decreased	SCFA production	Loss
<i>Clostridium</i> clusters XIVa and IV	Decreased	SCFA production, amino acid metabolism, bile acid metabolism	Loss
<i>Ruminococcus torques</i>	Decreased	Bile acid metabolism	Loss
<i>Bifidobacterium</i> spp.	Decreased	Amino acid metabolism, bile acid metabolism	Loss
<i>Bacteroides</i> spp.	Decreased	Complex carbohydrate metabolism, amino acid metabolism, bile acid metabolism	Loss
<i>Collinsella aerofaciens</i>	Decreased	Iron metabolism	Loss
Proteobacteria	Increased	Inflammation	Gain
<i>Escherichia coli</i> (adherent and invasive)	Increased	Inflammation	Gain
<i>Fusobacterium</i> spp.	Increased	Inflammation	Gain
<i>Ruminococcus gnavus</i>	Increased	Polysaccharides and mucin-degrading trans-sialidase	Gain
<i>Clostridium innocuum</i>	Increased	Associated with creeping fat	Gain
<i>Veillonellaceae</i>	Increased	SCFA production, inflammation	Gain
<b>Fungi</b>			
<i>Candida albicans</i>	Increased	Inflammation	Gain
Other <i>Candida</i> spp.	Increased	Inflammation	Gain
<i>Saccharomyces cerevisiae</i>	VAS	Inflammation (cross-reactive TCR in CD)	Gain
<i>Debaryomyces hansenii</i>	Increased	Decreased wound healing	Gain
<i>Malassezia restricta</i>	Increased	Inflammation	Gain
<b>Viruses</b>			
<i>Ackermannviridae</i>	Decreased	Unknown	Unknown
<i>Caudovirales</i> ( <i>Siphoviridae</i> , <i>Myoviridae</i> , <i>Podoviridae</i> )	Increased	Inflammation	Gain
<i>Microviridae</i>	Increased	Inflammation	Gain
<i>Helleviridae</i>	Increased	Inflammation	Gain
<i>Picornaviridae</i>	Increased	Inflammation	Gain

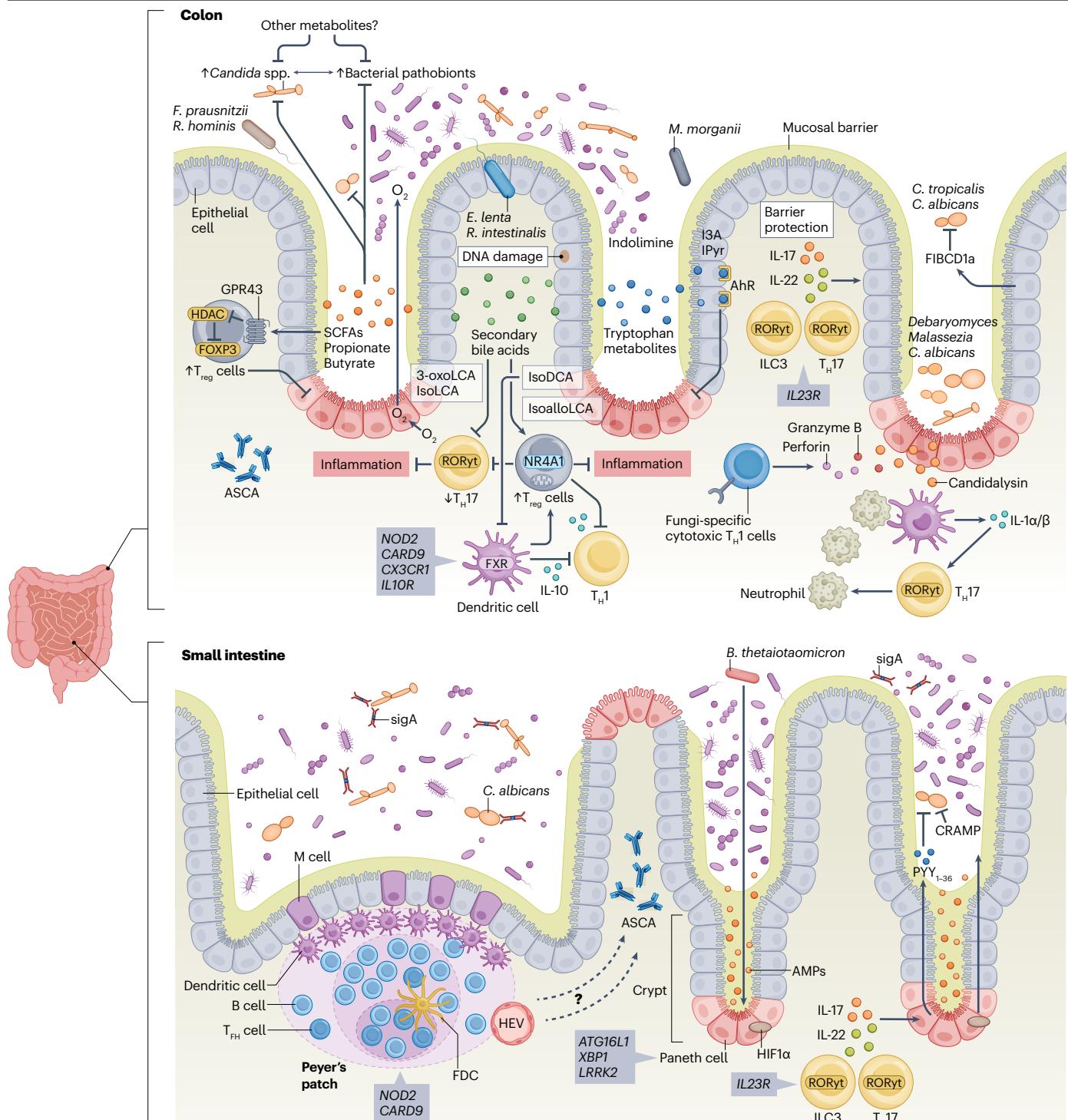
CD, Crohn's disease; IBD, inflammatory bowel disease; SCFA, short-chain fatty acid; TCR, T cell receptor; VAS, varies across studies.

across individuals govern the number of strains of each microbial species colonizing the gut of different people<sup>33</sup>, increasing complexity.

Advances in CRISPR-based bacterial engineering have allowed for precision manipulation of previously genetically intractable bacterial species and strains<sup>34,35</sup>. Findings based on comprehensive analysis and leverage of such new technologies reveal that key microbial and host-related microbiome functions are maintained by multiple species and strains<sup>36,37</sup>. Therefore, functional characteristics, as well as the ability of each individual microbial community to maintain stability, but not composition alone, are key to how the microbiome affects intestinal health.

## Fungi

Fungi are ubiquitous in the environment and present on all body surfaces, including the gastrointestinal tract<sup>38–50</sup>. Gut mycobiome composition is highly variable between and within individuals, as diet and environmental exposure (Box 1) change constantly<sup>51,52</sup>. Nevertheless, exploration of the mycobiome in IBD cohorts has determined the common presence of species from several genera including *Candida*, *Saccharomyces*, *Cladosporium*, *Malassezia*, *Pichia*, *Aspergillus* and *Penicillium*, with *Candida* species and *Candida albicans* found to be altered across multiple studies<sup>38–49</sup> (Table 1). *Malassezia* spp. and *Debaryomyces* spp. have also been linked to CD<sup>48,53</sup>. While both patients with CD and



inflammation<sup>46</sup>. Indeed, highly damaging *C. albicans* strains, producing the toxin candidalysin, have been identified in the gut of individuals with UC<sup>46</sup> (Fig. 1). Candidalysin induces intestinal inflammation by damaging epithelial membranes and activating the MAPK pathway<sup>54</sup>, leading to the recruitment of T helper 17 ( $T_{H}17$ ) cells, neutrophils and pro-inflammatory cytokines<sup>46,55,56</sup>. Genetic ablation of candidalysin leads to an inability to cause cell damage<sup>54</sup>.

**Fig. 1 | Microbiota-derived metabolites, immune and genetic mechanisms, and their effects on inflammation, intestinal biology and IBD.** The cross-kingdom microbiota regulates multiple immunological, tissue regenerative and microbial processes in the large and small intestine, to have a major impact on disease development and pathophysiology in both Crohn's disease and ulcerative colitis. Microbiota-derived secondary bile acids affect colon inflammation via regulating dendritic cells, neutrophils, macrophages, regulatory T cells ( $T_{reg}$  cells) and T helper 17 ( $T_{H17}$ ) cells. Indolimine, a tryptophan-derived metabolite, could potentially exacerbate colon inflammation by inducing DNA damage. Indole 3 acetic acid (I3A) and indole pyruvic acid (IPyr) ameliorate colon inflammation via activating the aryl hydrocarbon receptor (AhR). Isodeoxycholic acid (IsoDCA) promotes  $T_{reg}$  cells by antagonizing the farnesoid X receptor (FXR) in dendritic cells. IsoalloLCA induces FOXP3 expression by increasing mitochondrial reactive oxygen species levels and activating nuclear receptor 4A1 (NR4A1). Bacterial microbiota-produced short-chain fatty acids (SCFAs) induce  $T_{reg}$  cells via G protein-coupled receptor 43 (GPR43) and histone deacetylase (HDAC) inhibition. Candidalysin, a toxin produced by *Candida albicans* in a strain-dependent manner with high-damaging capacity in the gut of individuals with inflammatory bowel disease (IBD), aggravates inflammation through interleukin-1 $\beta$  (IL-1 $\beta$ )-dependent mechanisms. Fungal

antigen-reactive  $T_{H1}$  cells and antibodies against *Saccharomyces cerevisiae* mannan (ASCA) develop in patients with CD. Mechanisms that prevent fungal virulence and expansion of bacterial and fungal pathogens, such as secretory IgA (sIgA) antibodies that bind bacterial pathogens and *Candida* hyphae, SCFAs, hypoxic environment and antimicrobial peptides (AMPs) are impacted by IBD. Immune and cellular mechanisms that protect barrier integrity (for example, IL-17 and IL-22 production and mucus production) and inflammation (for example, IL-10 production) are also regulated by the cross-kingdom microbiota. Gain or loss of function mutation in genes involved in immune and epithelial function, and response to microbiota (arrow boxes) are associated with IBD. 3-oxoLCA, 3-oxolithocholic acid; *B. thetaiotaomicron*, *Bacteroides thetaiotaomicron*; *C. tropicalis*, *Candida tropicalis*; CRAMP, cathelicidin-related antimicrobial peptide; *E. lenta*, *Eggerthella lenta*; *F. prausnitzii*, *Faecalibacterium prausnitzii*; FDC, follicular dendritic cell; HEV, high endothelial venule; HIF1 $\alpha$ , hypoxia-inducible factor 1 $\alpha$ ; ILC3, group 3 innate lymphoid cells; LCA, lithocholic acid; M cell, microfold cell; *M. morganii*, *Morganella morganii*; PYY, peptide YY; *R. hominis*, *Roseburia hominis*; *R. intestinalis*, *Roseburia intestinalis*; ROR $\gamma$ t, retinoid acid-related orphan receptor- $\gamma$  transcription factor; ROS, reactive oxygen species;  $T_{FH}$ , T follicular helper.

Although deep-sequencing technologies have advanced our understanding of the gastrointestinal mycobiome, challenges persist in differentiating symbionts from transient fungal passengers and contaminants in faecal samples<sup>57</sup>. Low abundances pose a major challenge in retrieving fungal sequences and assembling fungal metagenomes from faecal metagenomics datasets. Internal transcribed spacer sequencing and culture-dependent methods remain primary approaches for exploring fungal composition in human faecal and mucosal samples. Traditionally, serological approaches have determined that antibodies against *Saccharomyces cerevisiae* mannan are elevated in individuals with CD, and their increased titres precede disease development<sup>58,59</sup>, linking fungi to IBD. Despite being challenging to implement in a clinical and translational research setting, recent approaches such as multi-kingdom antibody profiling<sup>60,61</sup> and antigen-reactive T cell enrichment<sup>62,63</sup> have provided deeper insights into the immune mechanisms involving gut fungi in human health and during IBD. In essence, multi-kingdom antibody profiling is a flow cytometry-based method that examines antibody responses to gut bacteria and fungi by utilizing faecal samples as microbial epitope sources, distinguishing fungi from bacteria based on their cell size and chitin content<sup>61</sup>. Antigen-reactive T cell enrichment, however, isolates and characterizes rare antigen-specific T cells directly from the blood through magnetic enrichment of CD154 $+$  cells, facilitating the study of naïve and memory repertoires specific to bacterial and fungal species without requiring prior knowledge of major histocompatibility complex alleles or antigenic epitopes<sup>63</sup>. Leveraging such immune-based approaches has already led to advances in the field and might provide an effective way of identifying fungal species and antigens involved in IBD immunopathology.

## Viruses

Viral particle enrichment and deep-sequencing analysis of viral DNA and RNA within faecal samples have revealed variations in virome composition among individuals with both UC and CD, marked by the presence of specific eukaryotic viruses and increased phage diversity<sup>64–66</sup>. An increase in eukaryotic viral reads has been described in individuals with CD compared with individuals without IBD, and an increase in

phage reads were described in individuals with CD compared with individuals without IBD or individuals with UC<sup>65</sup>. Caudovirales members *Siphoviridae*, *Myoviridae* and *Podoviridae* are increased in both CD and UC (Table 1). Furthermore, a clear difference in the virome between CD, and UC and non-IBD has been also described<sup>65</sup>. The majority of viral particles in human colon tissue belongs to phage families such as the double-stranded DNA *Caudovirales* and *Ligamenvirales* order, the single-stranded DNA phage family *Microviridae*, with elevation of the *Picornaviridae* family member *Enterovirus B*, in both UC and CD colon tissue. Notably, the presence of viruses can affect host immunity, inflammation or therapeutic outcomes<sup>65–68</sup>. Intestinal murine norovirus infection promoted intestinal pathology and a Paneth cell defect in mice with a mutation in *Atg16L1*, linking viral triggers with IBD for the first time<sup>69</sup>.

Altogether, technological advancements, coupled with multiomics approaches, strain-specific characterization and the use of microbial isolates from the human gut, have highlighted the critical role of specific gut bacterial and fungal species, as well as viruses, in health and IBD. Compositional changes in archaea<sup>42</sup> and protozoa<sup>70</sup> have been also reported in individuals with IBD; however, the functional role of these changes remains unclear. Current studies highlight the importance of strain-specific functional traits for bacteria and fungi that, depending on homeostasis or disease stage, influence gut health through physiologically active or immunoreactive metabolites and antimicrobial or immunomodulatory peptides.

## Microbiota-mediated mechanisms of intestinal homeostasis and IBD pathogenesis

Recent multiomics studies focusing on IBD have become a key resource and hypothesis-building framework for unravelling the molecular mechanisms governing gut microbial interactions and their influence on IBD pathogenesis and onset. Several categories of microbiota-associated metabolites – such as amino acids, bile acids, and SCFAs – consistently vary between patients with IBD and healthy individuals (Fig. 1). Longitudinal analysis of stool, biopsy, and blood samples collected every 2 weeks found that the metagenomic, metatranscriptomic, and metabolomic profiles of the microbiome

diverge over time for both individuals with and without IBD, but those with IBD have more pronounced changes<sup>15</sup>. Similarly, immune mechanisms that are protective during homeostasis become activated in IBD by microbiota and their metabolites, acting as key drivers of inflammation. In the following sections, we examine the interactions of microorganisms and their metabolites with host immunity.

## Immune mechanisms

**Microbiota and innate immune mechanisms of IBD.** Human genetic studies have shown that innate immunity plays a key role in the microbiota-mediated pathophysiology of IBD<sup>71</sup>. A high-throughput screening platform measured the innate immune responses of myeloid cells to 277 strains of bacteria isolated from the human gut<sup>72</sup>. Innate immune responses to gut bacteria were as strong as those towards pathogenic species of bacteria, but varied according to phylum or strain. Responses were dependent on Toll-like receptors 2 and 4, and in mice, bacterial stimulation induced colonic FOXP3<sup>+</sup> regulatory T ( $T_{reg}$ ) cells<sup>72</sup>. (Fig. 1). Genome-wide association studies in populations of individuals with IBD have defined risk and protective variants in several genes involved in microbial sensing, host–microbiota interactions and cytokine pathways, such as *CARD9* (ref. 73), *IL23R*<sup>73</sup> and *ATG16L1*, suggesting a potential role of microbiota and barrier immunity in the pathophysiology of IBD<sup>71,74,75</sup>. A certain risk variant (S12N; rs4077515) of *CARD9* fosters greater tumour necrosis factor (TNF) and interleukin 6 (IL-6) production by dendritic cells upon stimulation with depleted zymosan<sup>48,76</sup> or whole yeast (*Malassezia restricta*)<sup>48</sup> cells. The protective variant (S12NΔ11; rs141992399) leads to attenuated pro-inflammatory signalling through impaired interaction with the ubiquitin ligase TRIM62 upon stimulation with fungal cell wall ligands<sup>76</sup>. Experiential studies showed that *CARD9* is a critical mediator of systemic antifungal IgG antibody responses to *C. albicans* and demonstrated a role of gut commensal fungi in *CARD9*-dependent tuning of the human antibody repertoire<sup>61</sup>.

Defects in autophagy and loss-of-function mutations in autophagy genes are well-documented drivers of IBD. Polymorphisms in autophagy-related genes such as *ATG16L1*, X-box binding protein 1 and leucine-rich repeat kinase 2 are strongly associated with both UC and CD<sup>71,77–79</sup>, and are directly linked to antibacterial, antifungal and antiviral immunity. Individuals with variants in the IBD-associated genes autophagy-related 16 like 1 (*ATG16L1*) and *NOD2* show defective  $T_{reg}$  cell responses to outer membrane vesicles delivered by *Bacteroides fragilis*, which are required for protection from colitis<sup>75</sup>. Autophagy affects both the antimicrobial activity of Paneth cells and the number of intact secretory granules. Paneth cells regulate the intestinal microbiota through the release of antimicrobial peptides<sup>80</sup>. One of these recently identified antimicrobial peptides is peptide YY, which demonstrates selective antifungal activity against *C. albicans* hyphae<sup>81</sup>. Mice with variants of *Atg16l1* that are associated with an increased risk of CD, exhibit structural abnormalities in Paneth cells following murine norovirus infection – a defect also observed in individuals with CD who are homozygous for the *ATG16L1 T300A* variant<sup>69</sup>. Autophagy proteins prevent intestinal inflammation by supporting Paneth cell homeostasis and viability, by controlling endoplasmic reticulum stress pathways and secreting antimicrobial proteins to reinforce intestinal epithelial barrier function<sup>71,82,83</sup>. The interaction of Paneth cells with surrounding cell types such as  $\gamma\delta$  intraepithelial lymphocytes can orchestrate a switch from an antimicrobial to a carbohydrate transcriptional programme (transcription of enzymes and transporters of carbohydrate metabolism) by suppressing IL-22 production from

innate lymphoid cells in response to feeding<sup>84</sup>. Apoptosis inhibitor 5 produced by  $\gamma\delta$  intraepithelial lymphocytes protects Paneth cells from death during intestinal inflammation and acute viral infection in both mice and humans that carry the *ATG16L1* IBD risk allele<sup>83</sup>.

Tissue damage is involved in the inflammatory response mediated by immune and non-immune cells<sup>85–87</sup>. To recover tissue integrity, this process is naturally followed by tissue healing. These processes are inevitably affected by the microbiota. In colitis models, adherent IBD-associated microbiota can promote mucosal healing through induction and release of tumour necrosis factor-like ligand 1A (TL1A) by CX3CR1<sup>+</sup> mononuclear phagocytes (MNPs), which drives production of group 3 innate lymphoid cells and IL-22, both of which can promote mucosal healing during acute colitis<sup>88</sup>. CX3CR1<sup>+</sup> MNPs reduced  $T_{helper}$  cell expansion and promoted  $T_{reg}$  cell generation in the presence of normal microbiota, whereas disruption of the microbiota resulted in inflammatory  $T_{helper}$  cell responses<sup>89</sup>. Adherent-invasive *E. coli* that is enriched in individuals with CD promotes intestinal inflammation through propanediol dehydratase-dependent production of propionate and modulation of the IL-1 $\beta$ -producing CX3CR1<sup>+</sup> MNPs<sup>90</sup>. CX3CR1<sup>+</sup> MNPs are also essential for the initiation of innate and adaptive immune responses to intestinal fungi in a Syk-dependent manner, whereas genetic ablation of CX3CR1<sup>+</sup> MNPs in mice contributed to colitis that was rescued by antifungal treatment<sup>74</sup>. Loss-of-function mutation in *CX3CR1* in individuals with CD was associated with impaired antifungal antibody responses<sup>74</sup>. *C. albicans* strains with an increased ability to cause cell damage in the mucosa of individuals with IBD drive pro-inflammatory immunity in mouse models of colitis via IL-1 $\beta$  and NLRP3 inflammasome activation by candidalysin<sup>46</sup>. These findings lay the groundwork for pilot studies focused on the personalized targeting of fungal strains with these properties, within a rationally selected population of individuals with IBD. *Debaryomyces hansenii* recovered from mucosal samples of patients with CD<sup>53</sup> impairs colonic wound healing in mice via chemokine ligand 5 (refs. 53,74). Thus, bacteria and fungi can promote tissue damage or interfere with tissue healing processes, thereby affecting the pathophysiology of IBD. Genome-wide association studies have identified mutations in the *IL-23R* locus that are strongly associated with disease susceptibility<sup>91</sup>. IL-23 promotes colitis in mice through a  $T_{cell}$ -dependent mechanism<sup>92</sup>, and also induces  $T_{H17}$  cells that enhance barrier function via IL-17 (ref. 93), the production of which is enhanced by both bacteria and fungi<sup>94,95</sup>.

**Microbiota and adaptive immune mechanisms in IBD.** The  $T_{H17}$  response to specific strains is different between inflamed and non-inflamed conditions<sup>94</sup>. Profiling immune cells expressing the retinoid acid-related orphan receptor- $\gamma$  transcription factor (ROR $\gamma$ t) revealed that  $T_{reg}$  cells and group 3 innate lymphoid cells (ILC3), located predominantly in interfollicular regions, support the development of microbiota-specific  $T_{H17}$  cells, which fine tune the immune responses in the gut<sup>96</sup>. Single-cell RNA sequencing of  $T_{H17}$  cells identified genes governing pathogenicity, including *Gpr65*, *Plzp*, *Toso* and *Cd51*<sup>97</sup>. Colonization of bacterial strains from individuals with IBD exacerbated disease in a mouse model of colitis, which was dependent on  $T_{H17}$  and  $T_{reg}$  cells<sup>98</sup>. In addition to bacteria,  $T_{H17}$  responses also arise towards *C. albicans* in the human gut<sup>63,74,99,100</sup>, and are cross-reactive against other fungal species in patients with CD<sup>63</sup>. Nevertheless, it is still under debate whether microbiota-induced  $T_{H17}$  cells contribute to inflammation or healing in IBD, especially as targeting IL-17 has been ineffective in CD<sup>101,102</sup>. Effector  $T_{cell}$ s react to microbial ligands and have a role in the pathophysiology of both UC and CD<sup>103</sup>. In CD, commensal *C. albicans* and food-derived

yeasts such as *S. cerevisiae*, induce interferon- $\gamma$  (IFN $\gamma$ )-producing CD4 $^{+}$  T cells with cytotoxicity against intestinal epithelial cells<sup>62</sup> demonstrating that in addition to bacteria, fungal commensals and food-derived yeasts contribute to pathogenic CD4 $^{+}$  T cell responses in CD (Fig. 1). Aside from T cells, perturbations of the B cell compartment with expansion of bacteria-reactive IgG $^{+}$  plasma cells have been described in IBD, indicating a role in disease pathogenesis<sup>104–106</sup>. B cell-associated pathology in CD also involves cytotoxic T cells with specificity to gut fungal antigens, which are enriched in individuals with anti-*Saccharomyces cerevisiae* antibodies<sup>62</sup>. Although B cells can be involved in the pathology of IBD, they also play a crucial role in maintaining gut homeostasis by producing secretory IgA, the most abundant immunoglobulin in the gastrointestinal tract. Secretory IgA prevents direct contact between luminal factors, including bacteria, and intestinal epithelial cells<sup>107</sup>. Polyreactive IgA binds to a broad subset of bacteria with low affinity, including Proteobacteria, but its functional effects in IBD are still unclear<sup>108</sup>. Secretory IgA can also target fungi, with a high affinity for fungal hyphae<sup>60,109</sup>. Gut fungi such as *C. albicans* induce IgA class switch recombination or even activate antibody production systemically<sup>60,61</sup>. Altogether, these studies outline a dual role of microbiota-reactive B cells in IBD.

## Microbial metabolites

**SCFAs.** SCFAs such as acetate, propionate and butyrate are synthesized via gut microbial fermentation of dietary fibres and amino acids<sup>110</sup>, and are pivotal in sustaining intestinal homeostasis, facilitating innate and adaptive immune responses, and mitigating inflammatory reactions<sup>110</sup>. Previous research demonstrated that propionic and butyric acids contribute to colonic and peripheral T<sub>reg</sub> cell differentiation in mouse intestines through activation of the GPR43 fatty acid receptor<sup>111,112</sup>. Moreover, dietary fibre supplementation substantially elevates intestinal SCFA levels. Several ongoing clinical trials are exploring the potential of dietary interventions or fermentable fibre supplementation to modulate intestinal SCFA concentrations and alleviate symptoms of IBD.

In mice, butyrate contributes to differentiation of T<sub>reg</sub> cells<sup>113</sup>, which play a role in limiting inflammatory responses in the intestine<sup>114</sup>. T<sub>reg</sub> cell differentiation occurred via an extrathymic pathway dependent on CNS1, an intronic enhancer required for extrathymic but not thymic differentiation<sup>113</sup>. Propionate was also able to promote T<sub>reg</sub> cell differentiation, indicating that bacterial metabolites can mediate the balance of pro- and anti-inflammatory cells in the gut<sup>113</sup>. Antibiotic-mediated depletion of butyrate-producing species can reduce signalling through the intracellular butyrate sensor peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ), which prevents dysbiotic expansion of potentially pathogenic *Escherichia* and *Salmonella* species, highlighting the role of butyrate in inhibiting dysbiosis<sup>115</sup>.

A reduction in SCFA-producing bacteria has been observed in IBD, including *F. prausnitzii* and *R. hominis*<sup>115,21,116</sup>. Metabolomic analysis of samples from healthy individuals and patients with IBD showed reduction in butyrate, propionate and valerate/isovalerate during IBD dysbiosis<sup>115</sup>. In a dextran sodium sulfate mouse model, loss of GPR43 caused colitis, and binding of GPR43 by SCFAs was required for inflammation resolution<sup>117</sup>. SCFAs are also indirectly regulated through the NLRP1 inflammasome sensing. An activation mutation in the NLRP1A receptor increases IL-18 and IFN $\gamma$  production, which is associated with loss of beneficial, butyrate-producing species of the Clostridiales order<sup>118</sup>. In the same study, increased gene expression of *NLRP1* was found in patients with UC, providing a link among SCFAs, bacteria and

inflammation<sup>118</sup>. Finally, several SCFA have direct antibacterial and antifungal properties via intracellular acidification of bacterial and fungal cells<sup>119,120</sup>.

**Bile acids.** Bile acids can act as potent signalling molecules for host nuclear receptors. They can shape the gut microbiota, determining microbiota abundance, diversity and metabolic activity<sup>121</sup>. In newborns, primary bile acids drive the enrichment of bacteria expressing bile acid metabolism genes, which is important for bile acid tolerance<sup>122</sup>. In autoimmune cholestatic liver disease, in which bile flow from the liver is impaired, microbiota diversity is reduced<sup>123</sup>. Primary bile acids (cholic acid and chenodeoxycholic acid) undergo bacteria-mediated modification to generate a diverse range of secondary bile acids, which exert effects on metabolism and immune responses<sup>124,125</sup>. Emerging evidence suggests that specific secondary bile acids exert modulatory effects on immunity and colon inflammation through their interactions with nuclear receptors such as the farnesoid X receptor (FXR), ROR $\gamma$ , vitamin D receptor, Takeda G protein-coupled receptor 5 (TGR5) and nuclear receptor 4A1<sup>126</sup>. As IBD has an immunological component, it is plausible to hypothesize that these secondary bile acids may serve a regulatory role. An increase in primary bile acids, cholate and acylcarnitines, which are modified by microorganisms, was found to be characteristic for individuals with IBD<sup>15</sup>. It has been demonstrated that 3-oxolithocholic acid (3-oxoLCA), derived from lithocholic acid (LCA), inhibits intestinal T<sub>H</sub>17 cell differentiation via binding to ROR $\gamma$ , and isoalloLCA enhances T<sub>reg</sub> cell differentiation by binding to nuclear receptor 4A1 (ref. 127). Additionally, T<sub>reg</sub> cells induced by isoalloLCA suppress inflammation in colitis models. Screening of human stool samples containing high concentrations of 3-oxoLCA revealed that select human gut commensals produce T<sub>H</sub>17 cell-modulating bile acids, such as 3-oxoLCA and isoLCA. This screen identified 238 bacterial isolates belonging to 12 genera that could convert LCA into 3-oxoLCA, indicating a diverse range of bacteria can generate these secondary bile acids<sup>128</sup>. These bacteria were able to negatively regulate T<sub>H</sub>17 cell levels in vivo in mice through conversion of LCA into 3-oxoLCA and isoLCA<sup>128</sup>. In individuals with CD, levels of 3-oxoLCA and isoLCA were decreased; upregulated genes associated with T<sub>H</sub>17 cells and IL-17 in patients with IBD were found to have negative correlation with 3-oxoLCA and isoLCA (but not other bile acids), indicating that these secondary bile acids can contribute to IBD by altering the T<sub>H</sub>17–IL-17 signalling axis<sup>128</sup>.

Levels of these secondary bile acids and their corresponding microbiota genes were shown to be diminished in patients with IBD. One study revealed that isodeoxycholic acid promotes the generation of immunosuppressive CD4 $^{+}$  T<sub>reg</sub> cells through interaction with the FXR receptor in dendritic cells<sup>129</sup>. Alterations in bile acid composition have also been shown to regulate gut ROR $\gamma$  $^{+}$  T<sub>reg</sub> cell homeostasis via a vitamin D receptor-dependent mechanism<sup>130</sup>. Moreover, clinical studies found that patients with UC usually have reduced levels of secondary bile acids such as LCA, and LCA supplementation reduces intestinal inflammation in a TGR5-dependent mechanism<sup>131</sup>.

New bile acid conjugations with amino acids (phenylalanocholic acid, tyrosocholic acid and leucococholic acid) have also been identified through analysis of the metabolome in germ-free and specific pathogen-free mice<sup>132</sup>. These bile acid conjugates were enriched in patients with IBD; in particular, they were found to be present in a dysbiotic state from patients with CD, but not UC<sup>132</sup>. In mouse models, there was a strong positive correlation between the presence of *Clostridium bolteae* and conjugated bile acids, and further analysis found that certain strains of *C. bolteae* could synthesize phenylalanocholic acid.

In a cell-based system, bile acid conjugates stimulated the FXR receptor and expression of its target genes, indicating their mechanism of action<sup>132</sup>. Several additional amino acid-conjugated bile acids have since been discovered using reverse metabolomics approaches; similarly, they are increased in patients with CD, and are produced by bacteria belonging to the *Bifidobacterium*, *Clostridium* and *Enterococcus* genera<sup>133</sup>.

**Other microbial molecules and microbiota-mediated mechanisms.** The gut microbiota metabolizes tryptophan to various indole metabolites, including indole propionic acid and indole lactic acid<sup>134</sup>, which affect intestinal inflammation through aryl hydrocarbon receptor (AHR) signalling pathways<sup>134</sup>. A new class of genotoxic metabolites derived from tryptophan was recently discovered, termed indolimines, which can induce DNA damage<sup>135</sup>. The presence of a microbiota-encoded decarboxylase gene (aspartate aminotransferase) facilitates the biosynthesis of this class of metabolite. Furthermore, colonization by indolimine-producing gut bacteria (*Morganella morganii*) increased intestinal permeability – a phenotype frequently correlated with exacerbated colon inflammation in IBD<sup>135</sup>. Other bacterial species, including *Clostridium perfringens* and *Clostridium ramosum*, also produced small molecules that were able to induce DNA damage in cell-free assays and expression of the double-strand break marker γ-H2AX in epithelial cells<sup>135</sup>. DNA damage induced by these metabolites was caused by a mechanism that was independent of colibactin-mediated DNA damage driven by selected *E. coli* strains<sup>136</sup>, indicating the presence of multiple bacteria-associated genotoxic metabolites.

Depriving potentially harmful microbial species from access to essential nutrients through competition for common metabolites is another mechanism by which microbiota can exert beneficial effects in IBD. A consortia of 18 commensal bacterial strains isolated from healthy human stool was recently developed and shown to effectively suppress intestinal Enterobacteriaceae species, including *Klebsiella pneumoniae*, by competitively depleting gluconate<sup>137</sup>. Gluconate metabolism by species in this consortia involves a pathway of gluconate conversion to pyruvate and glyceraldehyde 3-phosphate through a series of enzymatic reactions involving gluconate dehydratase and additional enzymes. This pathway is not present in Enterobacteriaceae species commonly found in individuals with IBD. In mice, this consortia reduced inflammation and restored colonization resistance without impacting non-Enterobacteriaceae species. In individuals with IBD, the expansion of gluconate-utilizing Enterobacteriaceae is associated with disease whereas gluconate-metabolizing commensals correlate with gastrointestinal health<sup>137</sup>, underscoring the therapeutic potential of metabolic competition.

Recently, an innovative methodology known as ‘MetaWIBELE’ was developed to systematically identify over 340,000 protein families – half of which had not been functionally characterized previously – as potential bioactive agents in relation to gut inflammation<sup>7</sup>. This work also highlighted candidate microbiota proteins that are likely to interact with host immunity in the context of IBD. This study demonstrated that one protein containing a Von Willebrand factor domain contributed to biofilm formation in the presence of mucin. However, the direct impact of this protein on colon inflammation remains to be further investigated. This research not only establishes a novel analytical framework for exploring less well-characterized microbiota-encoded proteins, but also lays the groundwork for future investigations into the molecular mechanisms linking gut microbiota with IBD.

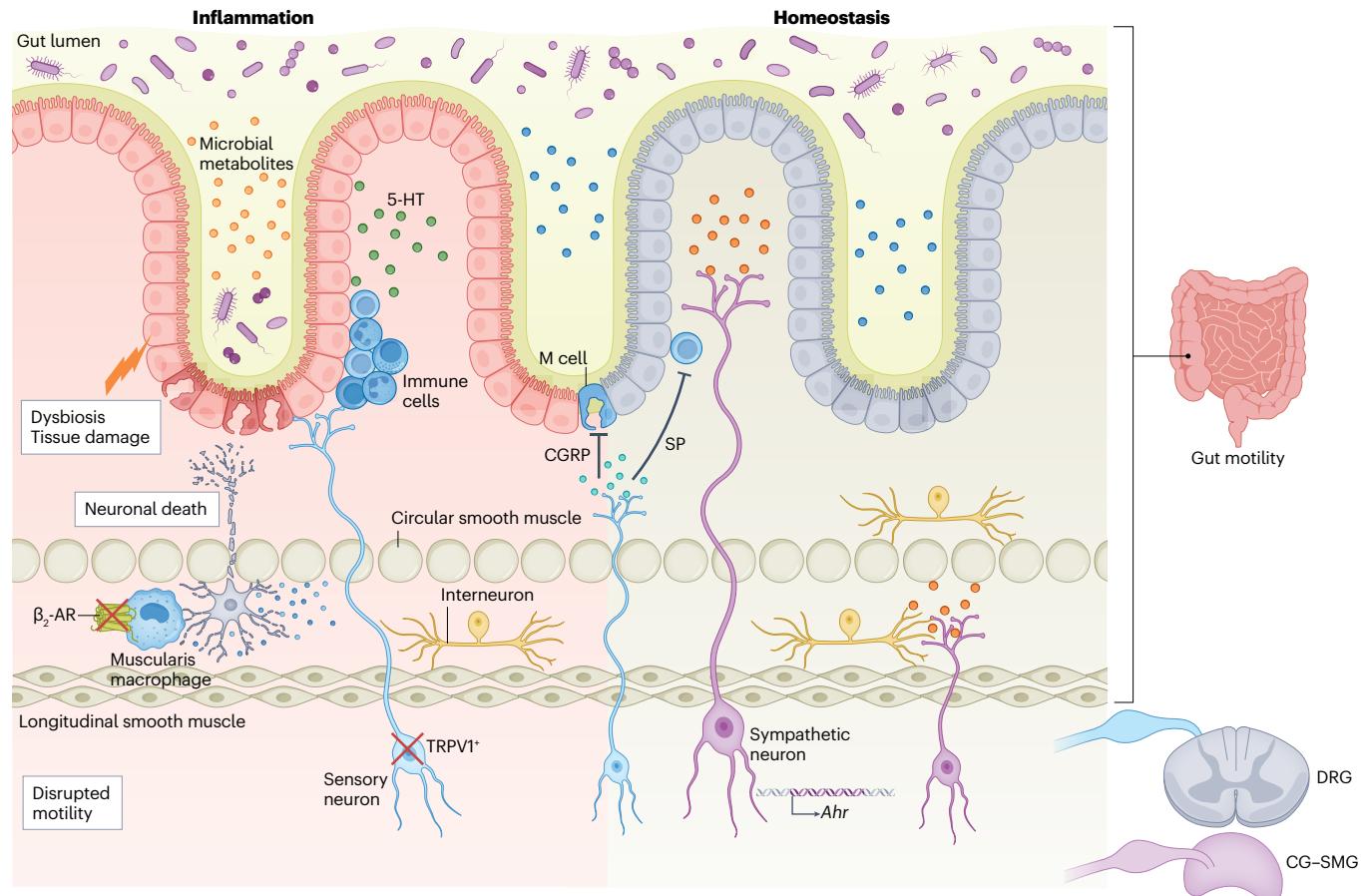
## Neuroimmune mechanisms of inflammation regulation

Acrosstalk between the gastrointestinal immune and nervous systems enables the sensing and rapid response to changes in the intestinal environment, including microbial and dietary cues, as well as tissue injury<sup>138,139</sup> (Fig. 2). The gastrointestinal tract is densely populated with enteric-associated neurons (EANs), which relay compositional information to other tissues to regulate gut function<sup>140</sup>. The intestinal microbiota are key regulators of the enteric nervous system, with neuronal detection of microbes eliciting transcriptional and functional responses<sup>141</sup>.

### Pain, nociception and the microbiota

Pain is a common symptom in individuals with IBD and the overabundance of EANs in inflamed regions of the bowel has been observed in IBD<sup>142</sup>. In transgenic mouse models (one with increased numbers of neurons and one with decreased numbers of neurons), the severity of colitis was found to correlate with the density of EANs in the gut<sup>143</sup>. The gut microbiome can modulate gut-extrinsic EANs. In a gnotobiotic mouse model, depletion of the gut microbiome resulted in increased expression of cFos, a marker of neuronal activity, indicating that the absence of microbiota can lead to increased sympathetic activity<sup>139</sup>. Transfer of specific bacterial species into germ-free mice resulted in a reduction in cFos expression, although this effect was not observed with segmented filamentous bacteria, *Akkermansia muciniphila* or *B. fragilis*, indicating that alterations in microbiome composition differentially regulate neuronal activity<sup>139</sup>. The presence of SCFAs produced by the microbiota reduced cFos activity, as did loss of bile acids, and investigation of additional microbiota-associated factors glucagon-like peptide-1 and peptide YY revealed that they also modulated sympathetic activity<sup>139</sup>. Microbial metabolites have also been shown to activate brainstem sensory nuclei, which can induce gut-specific stimuli, identifying a gut–brain–gut axis of communication<sup>139</sup>. In mice infected with *Citrobacter rodentium*, an immune response was triggered that led to the production of dietary-antigen-specific IgE antibodies in the intestine. Oral ingestion of bacterial antigens resulted in visceral pain via an IgE and mast-cell-dependent mechanism, thus identifying a link between the gut microbiome and pain<sup>144</sup>.

Nociceptive pain plays a role in many inflammatory diseases, including IBD. Nociceptors, which are specialized pain-sensing neurons, innervate peripheral tissues including the gastrointestinal tract; these neurons express transient receptor vanilloid 1 (TRPV1), a non-selective cation channel that can detect stimuli such as heat, capsaicin and inflammatory mediators<sup>145</sup>. In a mouse model of intestinal damage and inflammation, disruption of nociceptors through chemogenetic and adenoviral-mediated silencing or pharmacological TRPV1 nociceptor ablation resulted in increased inflammatory cell infiltration (including neutrophils), and defective tissue repair machinery<sup>146</sup>. These findings indicate that in a context-dependent manner, TRPV1 nociceptors play a protective role in the gut by regulating intestinal tissue damage, inflammation and repair<sup>146</sup> via the release of neuropeptides, such as calcitonin gene-related peptide and substance P. Disruption of TRPV1 nociceptors also altered intestinal microbiota composition in mouse models, including a decrease in *Bacteroides* and an increase in *Firmicutes* species<sup>146</sup>. Signals from intestinal microbiota in TRPV1-ablated mice promoted severe intestinal inflammation. Nociceptors release substance P, a neurotransmitter involved in pain signalling, which can also regulate the composition of the intestinal microbiota<sup>146</sup>.



**Fig. 2 | Neuroimmune regulation of intestinal barrier function, inflammation and pain.** Chronic pain is a key hallmark of inflammatory bowel disease. Microbial metabolites, immune cell- and neuron-produced mediators affect inflammation, neuronal function and the perception of pain. Communication between the gut microbiota and the immune system is further mediated by microbial metabolites, neurotransmitters and enteric-associated neurons. Pathobionts, intestinal barrier disruption and excessive immune activation

contribute to neuronal damage and pain in inflammatory bowel disease (left). Neurotransmitters produced by sympathetic and sensory neurons regulate gut motility or restrict bacteria invasion and inflammation at homeostasis via coeliac ganglion–superior mesenteric ganglion (CG–SMG) and dorsal root ganglion (DRG) (right). 5-HT, 5-hydroxytryptamine;  $\beta_2$ -AR,  $\beta_2$  adrenergic receptor; CGRP, calcitonin gene-related peptide; M cell, microfold cell; SP, substance P; TRPV1, transient receptor vanilloid 1.

Together, these findings indicate that nociceptor-mediated signalling regulates the gut microbiome through the release of neuropeptides, and that disruption of this pathway can cause microbial alterations and exacerbation of intestinal inflammation<sup>146</sup>.

### Microbiota effects on neuroimmunity and inflammation

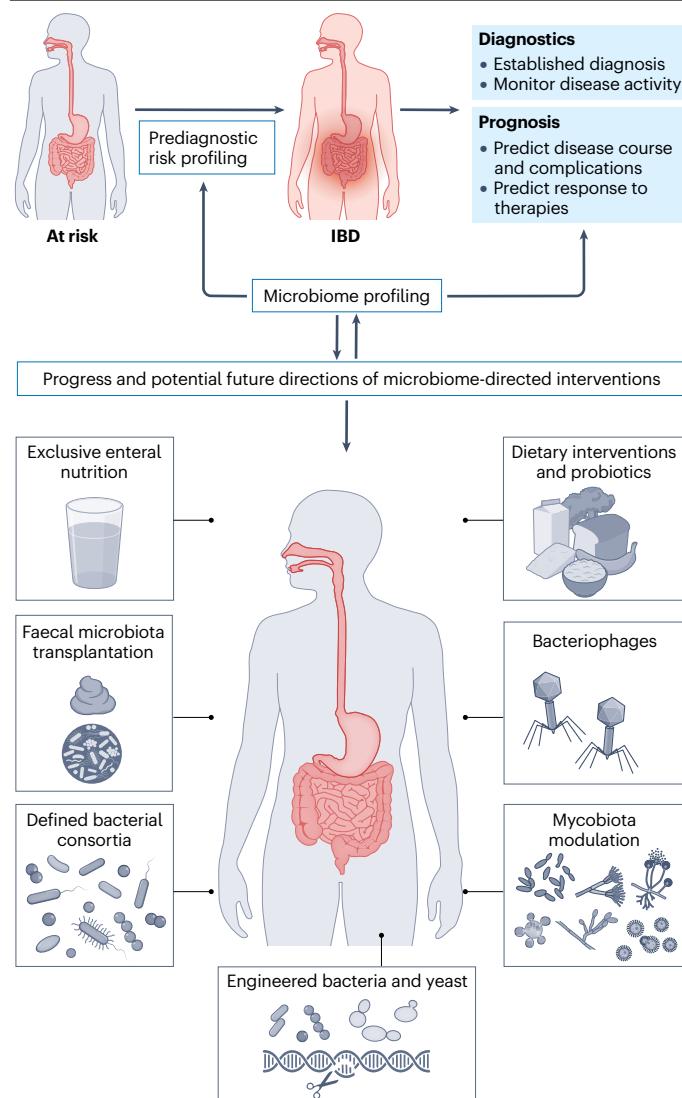
Pathogens and microbial metabolites can disrupt neuroimmune interactions. In mice, *Salmonella enterica* serovar Typhimurium infection caused loss of intestinal EANs and reduced motility through a pathway dependent on NOD-like receptor family pyrin domain-containing 6 (NLRP6) and caspase 11 (ref. 147). Following antibiotic treatment and recolonization of the gut with a healthy microbiota, EAN recovery was observed together with a return to pre-infection levels of neuronal activity<sup>147,148</sup>.

Adaptive immunity microbiota crosstalk can stimulate neuronal repair following tissue damage. The commensal *Staphylococcus aureus* induces T cells in densely innervated regions of skin. Upon injury, *S. aureus*-induced T<sub>H</sub>17 cells release IL-17A, which signals to sensory

neurons via the IL-17 receptor to initiate a transcriptional program associated with neuronal repair and local nerve regeneration<sup>135</sup>.

In mouse models, the presence of microbial communities in the colon has been linked to regulation of gut motility through expression of AHRs by EANs<sup>149</sup>. EANs can be induced by the microbiota to produce AHR, which acts as a biosensor and activates expression of genes that encode feedback regulators of AHR signalling and regulators of neuronal excitability, resulting in increased intestinal peristaltic activity, which is crucial for gut health<sup>149</sup>. Deletion of the AHR gene, or overexpression of its negative feedback regulator CYP1A1, resulted in reduced intestinal motility, an effect also observed in microbiota-depleted mice<sup>149</sup>. These findings provide a crucial link between the microbiota and gut motility. (Fig. 2)

Microbial metabolites can regulate activation of brainstem sensory nuclei with polysynaptic connections to the intestine, which may regulate gut sympathetic activity<sup>139</sup>. In mice in which TRPV1 or Nav1.8 sodium channel nociceptors were ablated, 16S rRNA gene sequencing revealed changes in the composition of the intestinal microbiota<sup>150</sup>.



**Fig. 3 | Therapeutic options and potential future directions for gut microbiome targeting in IBD.** Prebiotics, probiotics and synbiotics are among the microbiome-centred approaches used in individuals with inflammatory bowel disease (IBD), yielding mixed outcomes. Faecal microbial transplantation shows varied efficacy in ulcerative colitis, with remission rates of 24–53% in randomized controlled trials. Dietary interventions can alter the microbiome and, when personalized, can reduce inflammation in IBD. Bacteriophages, defined bacterial consortia, targeting of specific fungal strains and the use of engineered probiotics represent promising microbiome-based areas for future clinical investigation in IBD.

The neuron-depleted mice had fewer segmented filamentous bacteria, which were found to be important in mediating resistance against *S. Typhimurium* infection<sup>150</sup>. These findings provide a link between the neuroimmune system and intestinal defence against pathogens.

Serotonin is largely found within the gut and is regulated by the host microbiota. It is an important signalling molecule that exerts numerous effects on the gastrointestinal tract and other organ systems, with serotonin receptors located on enterocytes, EANs and

immune cells<sup>151</sup>. Dysregulation of serotonin has been linked to pathogenesis of several diseases, including irritable bowel syndrome<sup>152</sup>. Spore-forming bacteria from both mice and humans can promote biosynthesis of serotonin from colonic enterochromaffin cells through release of metabolites, indicating that they are modulators of gut serotonin levels<sup>153</sup>. It has also been shown that *R. gnavus* stimulates the production of serotonin in patients with irritable bowel syndrome through catabolism of dietary phenylalanine and tryptophan, resulting in activation of trace amine-associated receptor 1 (ref. 154). The increased production of serotonin resulted in increased gastrointestinal transit and colonic secretion, providing a mechanism through which microbial metabolites can contribute to serotonin dysregulation<sup>154</sup>.

Thus, the intestinal microbiota can tune the neuroimmune system, which in turn can elicit downstream effects to regulate gut function. The microbiome and microbial metabolites influence IBD symptoms through modulating pain and gut dysmotility. Conversely, neuronal factors and neuropeptides can affect the microbiota.

## Microbiota and IBD therapy

Current treatment options for CD and UC generally focus on targeting inflammation and promoting mucosal healing<sup>155</sup>. Potential therapeutic options specifically targeting the microbiome include antibiotics, prebiotics, probiotics, synbiotics (a combination of prebiotics and probiotics), faecal microbial transplantation (FMT) and dietary interventions. More recently, pioneering new biotherapeutics have shown potential for the treatment of IBD (Fig. 3).

## FMT

Although the pathogenesis of IBD differs between affected individuals, the possibility of introducing a healthy microbiota to patients with IBD has been investigated as a potential therapeutic option to treat IBD-associated dysbiosis. While FMT efficacy has been well documented in the treatment of *Clostridium difficile* infection<sup>156,157</sup>, randomized controlled trials of FMT in patients with UC have demonstrated modest effects on induction of remission<sup>158–162</sup>. Among five randomized controlled trials of FMT, varied by the donor or donors used, mode and duration of administration, four demonstrated a statistically significant benefit, resulting in remission rates of 24–53%. In a long-term follow-up of the FOCUS trial, of the 35 of patients who achieved remission with weekly FMT at 8 weeks, 34% remained in remission at 1 year, either with the aid of self-initiated FMT (three patients) or a change in diet (nine patients)<sup>163</sup>. To extend the durability of benefit from FMT, studies have also examined intermittent low-frequency FMT for maintenance or dietary alteration. In one study, 87% of patients could maintain clinical remission with FMT every 8 weeks for up to 48 weeks<sup>164</sup>, though fewer than half achieved endoscopic remission. An anti-inflammatory diet has been shown to maintain FMT induced remission for 1 year<sup>165</sup>. There is a paucity of data of this therapeutic option in CD. Both donor material and recipient-specific factors including engraftment probably play a part in the response to FMT<sup>158,166</sup>. In a randomized controlled trial of FMT in UC, the response rate for two distinct donors used was 10% and 39% respectively, suggesting variation in efficacy<sup>158</sup>. *Roseburia inulinivorans* and *Eubacterium hallii* enrichment, and an increase in faecal SCFAs was associated with remission, whereas an increase of *Escherichia* spp., *Fusobacterium* spp. and *Candida* spp. was associated with a lack of response. Increased *Candida* spp. abundance pre-FMT was associated with a clinical response, whereas decreased *Candida* spp. abundance post-FMT was indicative of ameliorated disease severity<sup>167</sup>. Although host factors strongly influence strain engraftment, a current

study demonstrates that only some strains within a species display a higher propensity for engraftment, even when introduced as part of multistain mixtures<sup>33</sup>. Thus, targeting the strain-level dynamics of the gut microbiome offers a strategy for replacing disease-associated strains with beneficial ones through sustained interventions that ensure long-term stability and engraftment. This approach could improve the efficacy of FMT and facilitate the development of rational microbial consortia for the treatment of IBD.

## Dietary interventions

Given the link between diet and the microbiome, and the presence of certain bacteria or fungi within particular foods, dietary intervention to manage IBD is an emerging therapeutic strategy. A link between higher intake of ultraprocessed foods, mostly consumed in a Western diet, and increased incidence of IBD has been identified<sup>168</sup>. There is also evidence that a high-fat, high-sugar diet can promote metabolic disease through interactions with the microbiota<sup>169,170</sup>. In mice, high-sugar intake led to an increase in *Faecalibaculum rodentium* that was dependent on ILC3s, which can exert both a protective and promoting effect on metabolic disease, suggesting a dual context-dependent role. The increase in *F. rodentium* displaced segmented filamentous bacteria that are inducers of intestinal T<sub>H</sub>17 cells, which exert a protective effect<sup>169</sup>.

A clinical trial comparing two microbiota-targeted dietary interventions (a high-fibre plant-based diet versus a high fermented food diet) in healthy adults used omics and immune profiling techniques to study both the host and the microbiome<sup>171</sup>. The trial identified key differences in the microbiome between the two groups: individuals on a fermented food diet had greater microbiota diversity, which coincided with a decrease in several markers of inflammation (including IL-6, IL-10 and IL-12b), and those on a high-fibre diet had stable microbiome diversity, an increase in microbiome-encoded glycan-degrading carbohydrate active enzymes, and a decrease in a specific subset of SCFAs<sup>171</sup>. These findings indicate that specific dietary interventions (namely increased intake of fermented foods) can alter microbiome composition and decrease intestinal inflammation.

There is conflicting evidence on the effect of dietary fibres in IBD. On one hand, dietary inulin fibre altered microbiota composition in a mouse model, resulting in a shift in levels of microbial metabolites, in particular bile acids, that was associated with type 2 inflammation<sup>172</sup>. This effect could also be induced by cholic acid delivery, thus mimicking the effect of inulin ingestion, and the deletion of the bile acid FX receptor reduced the effects of inulin. Mice colonized with human-derived microbiota displayed this effect. Moreover, deletion of the gene encoding a bile acid-metabolizing enzyme in *Bacteroides ovatus* abolished inulin-dependent type 2 inflammation in mice colonized with this bacterium<sup>172</sup>. These findings indicate that dietary fibre can induce type 2 inflammation in a microbiota-dependent manner through bile acid metabolism. Therefore, inulin intake might exert a negative effect, for example, on patients with coeliac disease or food allergies, where type 2 immunity plays a role. On the other hand, inulin-type fructans have been shown to induce clinical benefits in active UC: patients taking daily oral oligofructose-enriched inulin showed reduced colitis, increased colonic butyrate levels and increased abundance of SCFA-producing *Bifidobacteriaceae* and *Lachnospiraceae* species, indicating that fructans could be used as a potential therapy in UC<sup>173</sup>. A similar observation was noted in a recent study<sup>174</sup>, where beta-fructan replacement differentially introduced a pro-inflammatory or anti-inflammatory state based on the presence of active inflammation and the status of healing in patients with IBD. These examples

highlight the usefulness of preclinical models in determining the context-dependent effects of dietary additives and consideration of specific states of disease when interventions are carried in humans. These approaches should be integrated into clinical studies.

One of the most studied dietary interventions in IBD is exclusive enteral nutrition (EEN), which involves exclusive intake of a nutritionally complete liquid for about 8 weeks. EEN has demonstrated efficacy in paediatric patients with CD, with up to 80% going into remission after this diet<sup>175,176</sup>. It is not clear how EEN improves IBD symptoms, but there is evidence that it induces changes in the microbiome, with several studies showing that microbiome diversity is reduced on an EEN diet<sup>175,177</sup>. Although a reduction in microbiome diversity is often associated with an inflammatory state, in patients undergoing EEN this reduction in diversity is generally attributed to the limited components of the EEN diet compared with a regular diet<sup>178</sup>. Analysis of the faecal microbiota and metabolome of paediatric patients with CD after an EEN diet found that both were altered in patients who responded to therapy: a reduction in metabolites associated with IBD pathogenesis was observed, including the microbial metabolites cadaverine and trimethylamine, as well as a reduction in previously elevated levels of amino acids (serine, glycine and alanine)<sup>178</sup>.

## Prebiotics and probiotics

Trials of probiotics in IBD have yielded mixed results, which may, in some part, be due to the relatively modest effect of existing probiotics in achieving compositional and functional alteration in the microbiome. An eight-strain mixture containing *Lactobacillus* spp., *Bifidobacterium* spp. and *Streptococcus* spp. (VSL#3, Actial) was more effective in inducing remission in mild-to-moderate UC than placebo<sup>179</sup>, as well as efficacy in preventing recurrent pouchitis<sup>180</sup>. *E. coli* Nissle 1917 also demonstrated clinical efficacy in patients with UC, though the studies have been limited<sup>181</sup>. The prebiotics most studied in IBD include fructo-oligosaccharides and inulin, with inconsistent benefits and larger studies demonstrating no efficacy<sup>182</sup>. New studies hold promise for a conceptual change in the field by the introduction of rationally designed consortia of strains with an ability to engraft or prebiotics delivery in a condition-dependent manner, both discussed in the previous two sections.

## Biotechnology and biotherapeutics

Bacteriophages (phages) are self-replicating viruses that infect bacteria and use the bacterial cellular machinery to replicate. The ability of phages to target multidrug-resistant bacteria has raised the possibility of their use in IBD<sup>183</sup>. A five-phage combination targeting *K. pneumoniae*, a bacterium strongly associated with IBD exacerbation and severity, resulted in suppression of intestinal inflammation in a human gut IBD model<sup>184</sup>. Moreover, consumption of these phages by healthy volunteers was determined to have acceptable safety and showed that they accumulated in the lower gut<sup>184</sup>. As phages can be targeted precisely to specific membrane proteins, this makes them a promising tool for targeting specific bacterial strains.

VE202, a rationally defined live biotherapeutic product containing beneficial Clostridia strains, demonstrated safety and sustained colonization in healthy adults, paving the way for potential therapeutic trials in patients with UC (NCT05370885)<sup>185</sup>. A defined 18-member Firmicutes consortium (SER-301), which includes 10 strains has been tested for mild-to-moderate UC, with initially promising results in a phase Ib trial, but an inability to induce clinical remission in phase II<sup>186</sup>. The results from a phase IIa randomized controlled trial involving a six-member

defined consortium (MH002) in individuals with mild-to-moderate UC demonstrated a 17% improvement as measured by endoscopic Mayo scores as well as notable reduction of faecal calprotectin at week 8 when treated with the defined consortium compared with the placebo arm<sup>186</sup>. While it is still unclear whether these formulations provide benefit in individuals with IBD, additional studies are underway and data from both failed and successful clinical trials are being analysed to gain insights. Engineered probiotic yeast have demonstrated the ability to suppress intestinal inflammation in mouse models of IBD<sup>187</sup>. In this study, an *S. cerevisiae* strain was bioengineered to sense and respond to extracellular ATP, which can promote intestinal inflammation via the P2Y2 purinergic receptor. In the engineered strains, activation of P2Y2 was linked to apyrase, which breaks down ATP, generating a self-regulating feedback loop. In mouse models, these engineered yeast suppressed intestinal inflammation and reduced intestinal fibrosis and dysbiosis<sup>187</sup>.

## Microbiota determinants in response to biological therapies and inactivation of drugs in IBD

The evolving understanding of gut microbiota function and composition in IBD highlights its potential as a source of microbial biomarkers of disease states and response to therapies, with the potential to facilitate personalized treatment. In a prospective study, baseline stool and blood profiling of patients with moderate-to-severe CD or UC initiating anti-TNF, anti-IL-12/23 or anti-integrin therapy showed that microbial richness correlated with responses to therapy and abundance of bacterial species catalysing dehydroxylation of primary to secondary bile acids<sup>188</sup>. Specific serum immunoprofiling signatures correlated with bacterial diversity were indicative of remission likelihood upon anti-cytokine therapy and highlighted unique multiomic profiles for each therapeutic class<sup>188</sup>. Relative abundance of *Roseburia inulinivorans* and *Burkholderiales* sp. predicted response to anti-integrin therapy in UC and CD<sup>189</sup>. In a small paediatric IBD cohort, increase in  $\alpha$ -diversity and abundance of six bacterial clades, including *E. rectale* and *Bifidobacterium* spp., predicted response to anti-TNF therapy<sup>190</sup>. Notably, microbiota-based markers could also serve as predictors of disease course or relapse<sup>191,192</sup>.

In addition to bacterial products and metabolites, fungi and bacteriophages may also play a role in efficacy. In individuals with recurrent *C. difficile* infection, bacteriophage transfer during FMT can ameliorate disease<sup>193</sup> and low *C. albicans* abundance correlates with a positive FMT outcome<sup>194</sup>. In patients with UC, a high abundance of *C. albicans* pre-FMT was associated with increased bacterial diversity afterwards and increased success in patients with UC, suggesting that fungal ecology might play a role in bacterial engraftment<sup>167</sup>.

Whereas gut microbiota can metabolize and utilize IBD medications *in vitro*<sup>195</sup>, the impact of microbiota-mediated drug metabolism on the efficacy of IBD treatments in clinical settings remains underexplored. A recent analysis demonstrated that microbial acetyltransferases presence was correlated with an elevated risk of treatment failure when using 5-aminosalicylic acid, suggesting another mechanism through which gut microbiota affects IBD treatment and response<sup>196</sup>.

The gut microbiome also appears to regulate the efficacy of response to sulfasalazine, a prodrug used to treat IBD-associated peripheral spondyloarthritis. Patients who demonstrated a clinical response to sulfasalazine had a gut microbiome enriched in *F. prausnitzii* and levels of butyrate were elevated in the gut. In mice colonized with microbiota from responders, sulfasalazine was able to increase

faecal butyrate levels and limit colitis, indicating the role of the microbiome in determining response to therapy<sup>197</sup>.

## Conclusions and perspectives

Advances in technology and approaches to analyse the gut microbiome have elucidated new mechanisms through which bacteria, fungi and viruses are implicated in the pathogenesis of IBD. These findings have revealed that the role of the microbiota in IBD is multifactorial. It can exert beneficial effects through the release of metabolites, which tune the immune system, promote epithelial and tissue healing, and communicate with the nervous system. Conversely, bacterial and fungal metabolites, cell constituents and toxins can activate pro-inflammatory immune responses, cause tissue and neuronal damage, and negatively affect healing during flares and active disease. In this scenario, precision targeting of bacterial and fungal pathogens might provide new therapeutic opportunities. Interactions within and between these microbial kingdoms in the gut is another area worth attention: small molecules produced by bacteria and fungi as a means of cross-kingdom communication and competition might hold future therapeutic and diagnostic potential.

Preclinical models have been indispensable to the discovery of novel mechanisms as key biology is conserved across species. Nevertheless, disease modelling should be taken with caution. It should be considered that the divergence of effect between preclinical models and interventions in some cases may be a reflection of several factors. This includes the limited translatability of microbial association in mouse models to a human population. To address this aspect, models with rationally humanized microbiota have been introduced<sup>198</sup>, which can help avoid exaggerated causality claims<sup>198</sup>. Alternatively, models with evolutionarily adapted ‘wild-type’ rodent microbiota consisting of bacteria<sup>199</sup>, viruses<sup>199</sup> and fungi<sup>200</sup>, and rewilding<sup>201</sup> of mice<sup>202</sup>, can advance the modelling of outcomes and improve the translatability of preclinical interventions<sup>199</sup>. Similarly, limitations of human studies include small sample sizes lacking generalizability, incomplete adjustment for confounders (such as diet), poorly defined outcomes (symptoms versus objective inflammation) and heterogeneous populations where the effect may depend on baseline inflammatory state and specific characteristics of the microbiome. It is important for such confounding factors to be determined, quantified and reported in human intervention studies to infer mechanisms and effects.

On the clinical side, several aspects of how the microbiome intersects with clinical phenotype will be of high research priority in the field as outlined, for example, in the Crohn’s and Colitis Foundation Challenges priorities<sup>203,204</sup>. Cross-sectional studies in human cohorts have demonstrated the changes in microbiome in IBD, specifically in those with active disease. Longitudinal cohorts have begun to shed insights into how these changes may potentially be associated with disease progression and therapeutic response. Multiomic profiling of well-phenotyped cohorts provides insights into the functional perturbation and mechanisms of effect of microbial changes in IBD. These are an important component of precision medicine approaches for prognosis and treatment of IBD<sup>205</sup>. Furthermore, larger cohorts from diverse populations with consistently measured disease outcomes are essential to assess longitudinal effects. The study of the microbiome will also provide an important mechanistic insight into how the external environment modifies risk or outcomes of IBD, and explain the heterogeneity of the exposure–effect relationship<sup>203</sup>. Microbiome-directed interventions to improve outcomes of those with established IBD have yielded mixed results so far, with some promising dietary interventions

that induce beneficial changes in the microbiome. However, an important limitation in the field is the lack of ability for targeted and sustained modulation of the microbiome. In this aspect, engineered microbiota in combination with dietary interventions might provide new opportunities for the integration of microbiome-based therapies in the management of IBD.

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## Competing interests

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