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Gut microbiota and IBD: causation or correlation?

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Abstract

A general consensus exists that IBD is associated with compositional and metabolic changes in the intestinal microbiota (dysbiosis). However, a direct causal relationship between dysbiosis and IBD has not been definitively established in humans. Findings from animal models have revealed diverse and context-specific roles of the gut microbiota in health and disease, ranging from protective to pro-inflammatory actions. Moreover, evidence from these experimental models suggest that although gut bacteria often drive immune activation, chronic inflammation in turn shapes the gut microbiota and contributes to dysbiosis. The purpose of this Review is to summarize current associations between IBD and dysbiosis, describe the role of the gut microbiota in the context of specific animal models of colitis, and discuss the potential role of microbiota-focused interventions in the treatment of human IBD. Ultimately, more studies will be needed to define host–microbial relationships relevant to human disease and amenable to therapeutic interventions.

IBD, including crohn's disease and ulcerative colitis, affects ~3.1 million people in the USA and is increasing in incidence worldwide[1,2]. IBD is characterized by chronic immune-mediated intestinal inflammation that is driven by both genetic predisposition and environmental factors such as diet, antibiotic use and socioeconomic development[3].

A key role of the gut microbiota in the pathogenesis of IBD has long been postulated; however, definitive cause–effect mechanistic relationships have been challenging to prove outside of specific animal models. In particular, IBD has been associated with dysbiosis, defined as a decrease in gut microbial diversity owing to a shift in the balance between commensal and potentially pathogenic microorganisms[4–7]. Indeed, the clinical observation that IBD can respond to antibiotic treatment is consistent with the idea that intestinal bacteria contribute to the inflammatory response[8,9]. Other observations supporting a role for the gut microbiota in IBD include the predisposition of inflammation for anatomical regions with relative faecal stasis (terminal ileum and rectum), the effectiveness of faecal diversion

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as a treatment for Crohn's disease[10–12], and the rapidly increasing incidence of IBD globally associated with industrialization and accompanying alterations in diet and environmental exposures[13,14].

Although these associations are consistent with a role of the gut microbiota in IBD pathogenesis, the precise role of dysbiosis is less clear. Studies attempting to determine whether dysbiosis is truly causative or merely a consequence of inflammation have suffered from a number of limitations, making it difficult to draw definitive conclusions (BOX 1). In this Review, we will describe current associations between IBD and dysbiosis, the role of the gut microbiota in the context of specific animal models, and the potential clinical translation of microbiota-centered therapeutic approaches for human IBD.

Microbiota composition and IBD

Multiple studies have documented differences in the composition of the gut microbiota between patients with IBD and healthy individuals, particularly with respect to microbial diversity and the relative abundance of specific bacterial taxa. Both expansion of potential pathogens and global changes in composition (that is, increased or decreased abundance of indicator species) have been described. For example, the phylum Firmicutes — specifically *Faecailbacterium prausnitzii* — is often reduced in proportional abundance in the stool of patients with Crohn's disease[7,15–24], although studies focused on mucosal biopsies have questioned this association[25,26]. Conversely, members of the Proteobacteria phylum, such as Enterobacteriaceae[27,28], including *Escherichia coli*[19,29,30], are commonly increased in patients with IBD relative to healthy individuals. Differences in the composition of the gut microbiota have been documented even between members of the same family (including twins) who are discordant for IBD[31,32], suggesting that dysbiosis is primarily associated with disease state rather than environmental or genetic factors. Such cross-sectional studies do not provide information about the timing of dysbiosis relative to disease onset and, therefore, should be interpreted with caution particularly with regards to cause–effect relationships. For example, mucosal biopsies from twin pairs discordant for ulcerative colitis have shown a reduction in gut microbiota diversity in both siblings relative to unrelated healthy individuals, but the changes were more pronounced in the affected twin[33]. However, as the unaffected twins were not followed prospectively, it is not clear whether these findings reflected the gradual development of dysbiosis before the onset of IBD or, on the contrary, a lack of association between dysbiosis and clinical disease.

Studies of paediatric cohorts have attempted to address the temporal relationship between dysbiosis and inflammmation. Although there has been debate over the degree of dysbiosis in paediatric patients, particularly at the mucosal level, most studies have shown substantial differences between diseased and healthy individuals[34,35]. Thus, dysbiotic changes were described in stool and mucosal biopsies from newly diagnosed, treatment-naive children with Crohn's disease, suggesting that dysbiosis might precede clinical disease and develop independently of long-standing inflammation and/or medical therapy[36]. A prospective study of paediatric patients with Crohn's disease similarly concluded that dysbiosis reflected the presence and severity of inflammation; however, this study also found an independent association between dysbiosis and other factors such as diet and the use of antibiotics[37].

Thus, while changes in the gut microbiota might occur early in IBD and perhaps contribute to the onset of disease, over time environmental factors, including inflammation itself, probably further contribute to dysbiosis by altering the metabolic conditions in the gut (discussed later).

Although global changes in the microbiota of patients with IBD have been documented, strong evidence for the existence of specific pathobionts — commensal microorganisms that, under specific environmental or genetic influences, can cause IBD[38] — is limited. Enterobacteriaceae, and in particular certain strains of adherent–invasive *E. coli* (AIEC), have been associated with the ileal mucosa of patients with Crohn's disease[39] and have been proposed as potential pathobionts based on their ability replicate in epithelial cells *in vitro*[40]. *Mycobacterium avium* subsp. *paratuberculosis* has also been investigated as a potential cause of Crohn's disease owing to its ability to cause chronic granulomatous enteritis in sheep and cattle[41–44]; however, clinical studies have not borne out this hypothesis[45,46]. Similarly, a specific association between *Fusobacterium nucleatum*, ulcerative colitis and the development of colorectal cancer has been proposed based on the isolation of a highly invasive strain from patients with ulcerative colitis, but a clear cause-and-effect relationship has not been proven[47,48]. Pathobionts probably act in concert with the rest of the gut microbiota to cause disease, rather than as individual infectious agents, a possibility supported by observations in animal models (discussed later).

Although it is tempting to postulate an inciting role of the microbiota in IBD pathogenesis, it should be emphasized that the studies discussed so far describe associations and do not prove causation. In fact, evidence suggests that dysbiosis in IBD might, in large part, reflect the response of a complex microbial community to the environmental stress of intestinal inflammation. In the healthy gastrointestinal tract a radial oxygen gradient exists due to the diffusion of oxygen from the host mucosa into the gut lumen[49–51]. Accordingly, bacteria adherent to the colonic mucosa have higher oxygen tolerance and catalase expression relative to faeces-associated species[51]. As inflammation is an oxidative state, it might be expected to promote the outgrowth of aerotolerant taxa such as Proteobacteria and Actinobacteria. Indeed, the mouse pathogen *Citrobacter rodentium* has been shown to gain a fitness advantage by promoting epithelial aerobic respiration and increasing oxygenation of the mucosal surface[52]. Alternatively, several lines of evidence have shown that intestinal inflammation induces the production of small molecules that serve as terminal electron acceptors for facultative anaerobes such as Enterobacteriaceae[37,53]. Thus, metabolic alterations associated with inflammation and/or pathobiont colonization might act as microbial stressors and promote the outgrowth of dysbiotic species.

The nonbacterial microbiota and IBD

The virome and IBD

To date, most studies investigating the link between inflammation and the microbiota have focused on bacteria. However, the microbiome also includes fungi and viruses, and the role of these microorganisms in health and disease is being increasingly appreciated. Shotgun metagenomic analyses of viral particles isolated from faecal samples have shown that the gut virome is composed predominantly of bacteriophages[54–56]. Changes in bacteriophage

composition associated with IBD have been described, most notably an increase in *Caudovirales* bacteriophage sequences in ileal biopsy samples and intestinal washes from paediatric patients with Crohn's disease[57,58]. Importantly, expansion of *Caudovirales* bacteriophages was associated with a reduction in bacterial diversity. Through their diverse effects on bacteria — ranging from cell lysis to the transfer of genetic material encoding toxins or antibiotic resistance — phages can confer differential fitness on their hosts and influence the microbial composition of the gut (FIG. 1). Whether bacteriophages have a direct role in IBD pathogenesis, or merely reflect underlying dysbiosis remains to be determined. Similarly, a clear role for eukaryotic viruses in IBD has not been established[59].

The mycobiome and IBD

Antibodies directed against cell wall components of *Saccharomyces cerevisiae* have been associated with Crohn's disease since 1988 (REF. 60), but the clinical relevance of this finding remains unclear. Owing to technical challenges such as the misattribution of sequences and incorrect annotation of fungi in current genomic databases, relatively few studies have examined the role of fungi in IBD[61,62]. Fungal sequences can now be identified via culture- independent methods by PCR amplification of the small 18S ribosomal subunit or the internal transcribed spacer region[62,63], allowing for more comprehensive assessment of the mycobiome in health and disease.

A study of the stool-associated mycobiome showed an overall increased representation of fungi in paediatric patients with Crohn's disease compared with healthy individuals, however the same five taxa accounted for the majority of fungi in both groups[37]. Moreover, success ful therapy was associated with partial resolution of bacterial dysbiosis but did not lead to reduction in fungal colonization, putting into question the causative role of fungi in inflammation. Indeed, antibiotic treatment, which is commonly used in Crohn's disease, was independently associated with expansion of the same fungal taxa[37]. On the other hand, a study of adults with IBD did show differences in the relative abundance of specific fungi between patients with IBD and healthy controls[64]. Specifically, fungal dysbiosis in IBD was associated with an increased *Basidiomycota:Ascomycota* ratio, a decreased proportion of *Saccharomyces cerevisiae*, and an increased abundance of *Candida albicans*. This study further suggested that the inflammatory environment of Crohn's disease favours the expansion of fungi over bacteria (FIG. 1). Such a conclusion, however, should be interpreted with caution given the multiple confounders inherent in human research (BOX 1).

Studies of the mucosa-associated fungal composition have yielded similarly varied results. One study found that overall fungal diversity was higher in patients with IBD relative to healthy individuals, with several species detected only in Crohn's disease or ulcerative colitis samples, although no consistent pattern could be discerned[65]. This study also showed substantial differences in the fungal composition of stool and mucosal samples. Another study of paediatric patients failed to show statistically significant changes in the mycobiome of patients with Crohn's disease relative to healthy individuals, except for enrichment of the genus *Malassezia* which varied by geographical location[35]. The relative abundance of *Basidiomycota* and *Ascomycota* has also been examined in mucosal samples. In a study of paediatric patients, the *Basidiomycota*:*Ascomycota* ratio was found to be increased in

Crohn's disease compared with healthy individuals, similar to the findings above for stool[66]. On the other hand, in another study of adults with Crohn's disease, no discernible pattern with regards to these two phyla was seen[67].

Mechanistically, the involvement of fungi in IBD pathogenesis is plausible as a number of IBD susceptibility genes in mice and humans are involved in antifungal immune responses (for example, *CARD9*, *CLEC7A* and *RELA*)[68]. Mice lacking dectin-1 (encoded by *Clec7a*), the innate immune receptor that recognizes β-glucans in the fungal cell wall, have increased susceptibility to dextran sodium sulfate (DSS) colitis due to the expansion of opportunistic pathogenic fungi[69]. Similarly, *Card9*$^{-/-}$ mice have altered bacterial and fungal microbiota that cannot metabolize tryptophan into ligands for the aryl hydrocarbon receptor and, therefore, fail to upregulate IL-22, which is necessary for recovery from colitis[70]. More recently, S. *cerevisiae* colonization was shown to promote purine metabolism in mice, leading to elevated levels of uric acid, which has direct pro-inflammatory proper-ties[71]. Conversely, a protective role for the normal gut mycobiome (including *Malassezia* spp. and *C. albicans*) has also been postulated. For example, prolonged treatment of mice with the antifungal agent fluconazole leads to fungal dysbiosis characterized by the expansion of opportunistic species including *Aspergillus amstelodami*, *Epicoccum nigrum*, and *Wallemia sebi*. Mice enriched for these fungal organisms have worse outcomes of both DSS-associated and T-cell-transfer-mediated colitis with increased numbers of IFNγ and IL-17-secreting CD4^{+} T cells in the intestine[72]. Collectively, these animal models suggest that fungi might influence intestinal health and disease by suppressing the outgrowth of potential pathobionts, promoting immunoregulatory pathways, and modulating host metabolism. However, as illustrated by the human studies discussed earlier, establishing a direct causal relation ship between specific fungal species and health and disease remains challenging.

Microbial metabolites and IBD

Changes in the the composition of the gut microbiota lead to metabolite alterations that are likely to have a role in IBD pathogenesis. Through metabolomics, correlations between microbial composition and specific bacterial metabolic pathways can be established and the effects of small molecule (<1,500 Da) products on IBD pathogenesis assessed[73]. When the gut micro biota of healthy individuals and patients with IBD were compared, 12% of metabolic pathways were markedly different, compared with just 2% of genus-level clades[74]. Specifically, amino acid biosynthesis and carbohydrate metabolism pathways were reduced in the IBD micro-biome in favour of nutrient uptake, virulence and secretion pathways. Moreover, expression of genes related to oxidative stress, such as glutathione and sulfate transport, were increased[74]. These findings likely reflect the microbial response to the inflammatory intestinal environment of IBD and suggest that functional, rather than compositional, differences might be more informative when studying dysbiosis.

The microbial production of metabolites might affect the host in other ways that are relevant to IBD pathogenesis. For example, bile acid signalling via the nuclear farnesoid-activated X receptor (FXR, also known as bile acid receptor) has been shown to be protective in DSS and 2,4,6-trinitrobenzenesulfonic acid models of colitis by inhibiting NF-κB signalling[75]. As bile salt hydrolases (BSH) in intestinal bacteria play a key part in bile acid modification,

dysbiosis could have a direct effect on FXR signalling. Indeed, *in silico* analysis showed that the relative abundance of BSH in the gut microbiota was markedly reduced in patients with IBD compared with healthy individuals, and this reduction was most evident among Firmicutes from patients with Crohn's disease[76]. Consistent with these findings, levels of secondary bile acids are decreased in patients with IBD, particularly during flares[77]. Collectively, these data are consistent with a model in which impaired microbial enzymatic activity in IBD leads to altered bile salt metabolism and loss of anti-inflammatory signalling through FXR.

Another bacterial metabolic pathway with relevance to IBD is the production of short-chain fatty acids (SCFAs) through the fermentation of undigestable carbohydrates. SCFAs produced by specific clades of *Clostridia* spp. have been shown to augment regulatory T (T_{reg})-cell function in the intestinal mucosa through the activation of G protein-coupled receptors as well as via epigenetic effects leading to inhibition of histone deacetylase[78]. This process promotes restoration of immune tolerance and reduces inflammation in mouse models of colitis and asthma[78,79]. Efforts to target these pathways, either by altering the gut microbiota or through novel small molecule drugs are currently underway.

Host mucosal immune system: animal models

As discussed earlier, the gastrointestinal tract is populated by trillions of microorganisms that normally have a commensal or mutualistic relationship with their host. This healthy coexistence is maintained by a variety of immune mechanisms including secretion of mucus, immunoglobulin a (IgA), and antimicrobial peptides that shape the gut microbiota and prevent direct contact with the epithelium[80]. Conversely, the intestinal microbiota influences immune function in both health and disease. For example, germ-free mice have impaired immune development[81] and epithelial repair[82], while treatment with oral antibiotics can worsen the outcome of viral infections in mice[83]. In humans, the use of antibiotics in early childhood has been associated with increased risk of Crohn's disease, suggesting the microbiota might help set the threshold for immune activation versus tolerance[84]. Alternatively, antibiotics can potentiate the expansion of pathobionts such as *Clostridium difficile*, which is best managed by restoring intestinal microbial diversity using faecal microbiota transplantation[85]. Thus, the micro biota suppresses pathogens, promotes immune tolerance, initiates epithelial repair, and ensures the development of balanced immune cell subsets.

The healthy relationship between host and gut micro-biota is tested at times of mucosal injury when changes in immune activation and microbiota composition are likely to take place[80]. Given its vast surface area and constant exposure to the environment, the intestinal epithelial barrier is susceptible to damage by pathogens, ischaemia and environmental toxins. Numerous animal models of colitis have explored the diverse ways in which such insults lead to chronic inflammation in genetically predisposed animals, revealing complex immune– microbial interactions. Notably, although animal models have greatly advanced our understanding of IBD pathogenesis, they have inherent limitations (BOX 2). Nevertheless, these systems have enforced the notion of ‘dysregulated’ immunity as a key driver of IBD and have established diverse and context-dependent roles for the gut micro-

biota in health and disease. In the following sections the issue of causation versus correlation of dysbiosis will be considered in the context of specific IBD animal models.

IL-23 signalling, T_H17 cells and type 3 innate lymphoid cells

Genome-wide association studies (GWAS) have established a role for IL-23 signalling in the pathogenesis of several immune-mediated diseases including IBD[86]. Polymorphisms in genes encoding the β subunit of IL-23 (IL-12p40), its receptor, and downstream signalling molecules (STAT3 and JAK2) have been implicated[87], while blockade of IL-12p40 is an effective treatment for Crohn's disease according to a phase III clinical trial[88]. IL-23 is secreted by intestinal dendritic cells (DCs) and promotes the expansion of type 17 T helper (T_H17) cells and type 3 innate lymphoid cells (ILC3s). Development of these immune subsets is driven by the transcriptional factor RORγt and their signature cytokines (IL-17, IL-21 and IL-22) have key roles in mucosal defenses and immunopathology[89,90].

IL-23 signalling is central in the adoptive T-cell transfer and *T-bet*$^{-/-}$*RAG*$^{-/-}$ ulcerative colitis (TRUC) models of spontaneous colitis[91]. In the adoptive T-cell transfer model, naive $CD4^+$ donor T cells induce spontaneous colitis when transferred to SCID (severe combined immunodeficiency) or *Rag*$^{-/-}$ recipients lacking adaptive immunity[92]. Inflammation in this model is mediated by T_H17 cells and T cells of a mixed phenotype capable of secreting both IL-17 and type 1 cytokines (such as IFNγ)[93,94]. TRUC mice, on the other hand, lack both adaptive immunity and T-bet-dependent components of innate immunity (including ILC1 and $NKp46^+$ ILC3s). In this setting, IL-23 signalling leads to expansion of $NKp46^-$ ILC3 that mediate inflammation via IL-17 and IL-22 secretion[91].

A key feature of the adoptive transfer and TRUC models is their dependence on the microbiota, as germ-free animals do not develop colitis. In the adoptive T-cell transfer model, several specific micro organisms have been implicated, including *Helicobacter muridarum*, *Helicobacter hepaticus* and segmented filamentous bacteria (SFB); however, in most cases, colonization with a defined cocktail of specific pathogen- free (SPF) bacteria is also required for colitis to develop[95–97]. Conversely, co-colonization with the human symbiont *Bacteroides fragilis* reverses the inflammatory effects of *Helicobacter hepaticus* by promoting T_{reg}-cell develop-ment[98,99]. Similarly, *Klebsiella pneumoniae* and *Proteus mirabilis* cause colitis in TRUC mice, but only in the presence of the endogen ous microbiota[100]. Thus, the inflammatory potential of individual pathobionts is ultimately determined by the overall composition of the gut microbiota. Moreover, TRUC mice can transmit colitis even to wild-type animals, suggesting that the inflammatory environment can condition the gut microbiota to cause disease even in the absence of host risk factors[101].

Regulatory T cells and immune tolerance

T_{reg} cells are a distinct subset of helper T cells defined by the transcription factor FOXP3 and anti-inflammatory cytokine IL-10. As with T_H17 cells, T_{reg} cells are induced by transforming growth factor (TGF) β, accumulate at mucosal surfaces, and differentiate under the influence of commensal organisms and environmental signals[81,102,103]. In humans, mutations in *FOXP3* lead to chronic enteritis[104], whereas blockade of SMAD7, an inhibitor of TGFβ, improves inflammation by augmenting T_{reg}-cell function[105,106]. Similarly, *IL10*

polymorphisms have been associated with human IBD in GWAS, and congenital IL-10 deficiency leads to severe childhood colitis[107,108]. Not surprisingly, spontaneous colitis in *Il10*$^{-/-}$ mice depends on the gut microbiota and is driven by unopposed T_H17 cells[109,110]. Conversely, specific microbial species — including *Bacteroides fragilis*[98,99] and a consortium of human-derived *Clostridia* strains[111] — can alleviate inflammation in several colitis models by promoting T_{reg}-cell development (FIG. 2). Thus, the relative abundance of T_H17 and T_{reg} cells is a key factor in determining intestinal inflammation versus tolerance. By differentially promoting the development of these immune subsets, the gut microbiota have an important role in maintaining or disrupting intestinal homeostasis.

Microbial sensing, the inflammasome and epithelial repair

The complex relationship between the gut microbiota and intestinal inflammation is further illustrated by innate models of colitis. Innate immune cells detect pathogenic and commensal bacteria via a conserved set of pattern-recognition receptors that are linked to NF-κB activation, inflammasome assembly, and epithelial repair pathways (FIG. 2). A number of these receptors — most notably NOD2, NLRP3, and several Toll-like receptors (TLRs) — have been implicated in the pathogenesis of IBD by GWAS[112–118]. Animal models involving NOD-like receptor (NLR) or TLR signalling defects have revealed a complex and context-dependent role of innate immunity in colitis, ranging from protective to pro-inflammatory.

MYD88 is a central adaptor for signalling through most TLRs, as well as the IL-1 and IL-18 receptors, and disruption of this protein leads to profound innate immune dysfunction[82]. Administration of DSS to *Myd88*$^{-/-}$ mice results in severe and lethal colitis which, in contrast to the models discussed earlier, is not driven by the gut microbiota[82]. Thus, germ-free *Myd88*$^{-/-}$ mice are not protected from DSS, and in fact antibiotic treatment of wild-type animals exacerbates disease severity[82], consistent with a protective role for the gut microbiota. Indeed, bacterial sensing and signalling through TLRs is critical for epithelial repair by promoting secretion of tissue-protective factors, inhibiting apoptosis, and recruiting stromal and myeloid cells to the colonic crypts[119-124]. Thus, the normal gut microbiota is essential for restoring homeostasis after acute mucosal injury.

A number of other mouse models have linked TLR and NLR signalling to epithelial repair and homeostasis after DSS injury. For example, absence of NF-κB activation in *Nemo* (also known as *Ikbkg*)$^{-/-}$ or *Ikk1/2* (also known as *Chuk–Ikbk*)$^{-/-}$ mice results in spontaneous colitis characterized by increased epithelial apoptosis[125]. Similarly, inflammasome disruption via deletion of NLRP3 (linked to IBD in GWAS), NLRP6, PYCARD, caspase 1, or IL-18 leads to impaired epithelial repair and increased cancer susceptibility[126–130]. However, in most of these mouse models, defects in innate immunity also lead to the outgrowth and translocation of pathogens that contribute to the overall disease phenotype and respond to antibiotic treatment[125,128]. For example, DSS colitis in NLRP6-deficient mice is complicated by the outgrowth of Prevotellaceae species, which can exacerbate disease when transferred to wild-type animals[130]. Similarly, *Myd88*$^{-/-}$ mice develop severe colitis and bacteraemia when colonized with the pathogen *Citrobacter rodentium*[131,132]

(FIG. 2). Thus, sensing of the gut microbiota by innate immune cells maintains intestinal homeostasis by both limiting bacterial growth and promoting epithelial repair.

Paneth cells, autophagy and Crohn's ileitis

Autophagy is a conserved process of targeted degradation of cytoplasmic pathogens and cellular components via the formation of a double-membrane vesicle[133]. This pathway has been implicated in the pathogenesis of Crohn's disease by GWAS, which have identified susceptibility loci in the *ATG16L1* and *IRGM* autophagy genes[134,135]. ATG16L1 has been linked to NOD2 activation[136,137] and plays a key part in the function of Paneth cells, specialized epithelial cells located in the intestinal crypt base[138]. Paneth cells shape the gut microbiota via secretion of antimicrobial peptides[139]; conversely, dysfunction of these cells has been associated with Crohn's disease[140]. Cadwell *et al.*[141] have shown that mice hypomorphic for ATG16L1 develop Paneth cell defects upon infection with a chronic strain of murine norovirus (MNV). Subsequent administration of DSS to such MNV-infected mice triggers Crohn's disease-like colonic lesions in a microbiota-dependent manner. This model provides an elegant example of the convergence of genetic, environmental and microbial factors to produce the clinical phenotype of Crohn's disease.

Paneth cell dysfunction and dysbiosis have also been implicated in the $TNF^{\Delta ARE}$ model of Crohn's disease ileitis[142]. $TNF^{\Delta ARE}$ mice have dysregulated TNF expression due to deletions in the AU-rich elements (ARE) of *Tnf*, leading to chronic ileal inflammation[143]. Ileitis in conventionally reared $TNF^{\Delta ARE}$ mice is microbiota-dependent and can be attenuated by antibiotic treatment. Remarkably, $TNF^{\Delta ARE}$ mice reared in an SPF environment, develop discrete gradients of disease activity ranging from normal to severe ileitis. These clinical phenotypes correspond to distinct degrees of dysbiosis and Paneth cell dysfunction in individual mice. Moreover, the gut microbiota from inflamed, but not from healthy, $TNF^{\Delta ARE}$ mice can transmit ileitis and Paneth cell defects to germ-free $TNF^{\Delta ARE}$ recipients, pointing to a direct causal relationship between dysbiosis and inflammation.

IgA-coated taxa in the gut microbiota as possible pathobionts in IBD

IgA is the predominant antibody isotype at mucosal surfaces and exerts protective and immuno-regulatory effects by modulating the composition of the gut microbiota[144]. IgA coating of intestinal bacteria can distinguish colitogenic (IgA^+) from commensal (IgA^-) species, and IgA^+ bacteria identified in this manner include known pathobionts (Prevotellaceae, *Helicobacter* and SFB) capable of mediating DSS-induced colitis when transferred to SPF mice[145]. Moreover, IgA^+ bacteria isolated from patients with IBD, but not from healthy individuals, led to severe DSS-induced colitis when transferred to SPF mice[145]. A major feature that dis tinguished colitogenic IgA^+ from non-colitogenic IgA^- bacteria was the ability of the former to penetrate the mucus layer and trigger T-cell-dependent high-affinity IgA production (FIG. 2). Thus, identifying colitogenic bacteria based on IgA coating could eventually lead to a personalized treatment approach for IBD. These findings further suggest that IgA has an anti-inflammatory role by selectively neutralizing colitogenic bacteria. Consistent with this interpretation, IgA deficiency has been associated with a 3.9 and 5.7 prevalence ratio for ulcerative colitis and Crohn's disease, respectively[146].

Human IBD: evidence from clinical studies

As discussed in the preceding sections, dysbiosis in patients with IBD has been well-documented. Moreover, the microbiota have a variety of roles in IBD models of intestinal inflammation, ranging from protective to causative. As many such models have been informed by GWAS data and are based on knockouts of human susceptibility genes, one might speculate that bacteria, fungi and viruses have similar roles in human IBD. However, as discussed in BOXES 1, 2, results from animal studies have a number of limitations and must be interpre ted with caution, especially with regards to their relevance to human disease. The question of whether dysbiosis precedes the development of IBD and sets the inflammatory process in motion, or merely reflects the altered immune and metabolic environment of the inflamed mucosa, remains to be answered. Although a number of human participant studies support a causal role of dys biosis in IBD pathogenesis, conclusions are limited by the lack of prospective data.

IBD preferentially affects intestinal regions with the highest abundance of bacteria, and both faecal diversion and antibiotics can be effective in the management of Crohn's disease[10–12]. Individuals with active Crohn's disease who undergo ileocolonic resection and placement of a diverting ileostomy often have a normal neoterminal ileum 3–6 months after surgery[11,147]. Moreover, infusion of the proximal ileum effluent into the excluded distal ileum can lead to disease recurrence, although the precise factor(s) mediating inflammation have not been determined[147]. Of note, faecal stream diversion is not universally effective and can itself be colitogenic by depriving colonocytes of SCFAs normally produced by resident bacteria[148].

The possibility that inflammation in IBD might be driven by the gut microbiota has informed a number of clinical approaches aimed at correcting dysbiosis by dietary or microbial interventions. Examples include the use of probiotics, antibiotics, defined enteral nutritional therapy (ENT), and faecal microbiota transplantation (FMT). Probiotics are mixtures of bacteria or yeasts with perceived beneficial health effects, utilized to restore gut microbial balance[149]. To date, evidence for the efficacy of probiotics in the treatment of IBD is equivocal[149]. The most compelling findings come from post-surgical patients with ulcerative colitis who have undergone ileal pouch-anal anastomosis. Such patients are predisposed to inflammation of the ileal pouch (pouchitis) and probiotics have been shown to be effective in preventing this complication following successful antibiotic treatment[149,150]. However, basic questions regarding the optimal composition of probiotics, timing of administration and durability of the response remain unanswered.

Antibiotics can alleviate inflammation in various animal models of colitis; however, their effectiveness in human IBD has been limited. Two meta-analyses of randomized controlled trials did show a statistically significant benefit of antibiotics relative to placebo in the treatment of both Crohn's disease and ulcerative colitis (relative risk of remission or relapse between 0.62 and 0.85 (REF. 8); odds ratio 1.35 to 2.17 in favour of antibiotics[9]. Moreover, several studies have shown that broad-spectrum antibiotics improved rates of steroid-free remission in ulcerative colitis[151–153]. Given the phenotypic heterogeneity of IBD and the diversity of human populations, it is not surprising that inconsistent outcomes of antibiotic treatment have been reported. Indiscriminately targeting the gut microbiota with broad-

spectrum antibiotics probably depletes beneficial, as well as pathogenic, microorganisms with potentially unpredictable consequences. As with probiotics, future antibiotic regimens might have to be tailored to individual patients based on their specific gut microbiota composition and genetic makeup.

Diet has been shown to have a major effect on the composition of the gut microbiota by altering its functionality and metabolism at the genomic level[154]. Epidemiological evidence points to a role of diet in the pathogenesis of IBD, and dietary modification has long been used as therapy for Crohn's disease[155]. ENT with elemental, semi-elemental, or polymeric formulas can be used as first-line therapy for the induction of remission in Crohn's disease and has been associated with both clinical improvement and mucosal healing[156–161]. Although the mechanism of action of ENT has not been well defined, hypotheses include reduction in luminal antigens secondary to food exclusion and/or modulation of the gut microbiota and its metabolome. Several studies have shown a change in faecal microbiota composition following ENT therapy[37,162,163], as well as functional changes leading to increased levels of anti-inflammatory SCFAs in children with Crohn's disease[164]. ENT was effective in inducing remission in paediatric patients with Crohn's disease, and was associated with rapid changes in gut microbiota composition[37]. Another study further showed that ENT was associated with reduced numbers of T_{reg} cells in the lamina propria, reflecting the resolution of intestinal inflammation[165]. Notably, favourable therapeutic outcomes of ENT that we and others have reported was associated with initial shifts in microbial composition towards even greater dysbiosis relative to healthy individuals[37,166]. These observations highlight the need to further explore the effects of diet on microbial composition and disease activity, and further point to the complex relationship between dysbiosis and IBD.

FMT has been explored as a strategy to correct dysbiosis. The success of FMT for the treatment of refractory *Clostridium difficile* infection[167] has generated strong interest in using this approach in IBD. However, clinical results so far have been varied, possibly reflecting the higher complexity of IBD compared to *C. difficile* colitis[168–171]. Two randomized, placebo-controlled trials for the treatment of ulcerative colitis showed that FMT was markedly more effective than placebo in inducing clinical and endoscopic remission in ulcerative colitis in adults[172,173]. However, one other recent randomized, placebo-controlled trail failed to show a similar benefit of FMT[174]. Even fewer studies have explored FMT for the treatment of Crohn's disease and no randomized-controlled clinical trials have yet been completed although several are ongoing[175,176]. In children, FMT has shown clinical benefit in a small cohort of patients with Crohn's disease[177], whereas a pilot study of FMT in adult refractory Crohn's disease resulted in high rates of clinical remission and clinical improvement[178]. Overall, data regarding FMT are scarce and questions remain regarding the safety and durability of this approach (short-term and long-term), particularly in immunosuppressed patients; the most effective mode of administration; and how to select appropriate donors and recipients. Larger randomized controlled trials are necessary to better define the role of FMT in the treatment of IBD.

Conclusions

In this Review, we have summarized human microbiome and metabolome associations with IBD, provided an overview of animal models with a focus on host–microbial interactions and their effect on mucosal homeostasis, and discussed the potential therapeutic role of the gut micro-biota in IBD management. Our analysis seems to suggest a greater effect of the gut microbiota on disease phenotype and activity in mice than in humans receiving microbial-based therapies. What is the basis for this difference? One interpretation is that the micro biota plays, at most, a limited part in the pathogenesis of human IBD and dysbiosis is simply a marker of disease (BOX 3). Such a conclusion is supported by a longitudinal analysis published in 2017 of faecal microbiota from patients with IBD, which failed to show a correlation between the degree of dysbiosis and Crohn's disease severity[24]. It should be noted, however, that the authors used faecal calprotectin levels as a marker of disease activity and, therefore, might have underestimated the severity of ileal inflammation. In fact, the same study showed that recent steroid use (which probably reflected disease exacerbation) did correlate with increased microbiome instability. Similarly, a study of paediatric patients with IBD showed a significant correlation between microbota composition and disease severity, with resolution of dysbiosis in patients responding to anit-TNF therapy[179]. Nevertheless, a clear cause-effect relationship between dysbiosis and human IBD reamins to be definitively established.

We believe in a nuanced interpretation of the differences between mouse and human studies. First, responses to the gut microbiota are likely to differ between mammalian species. Second, mice are genetically homogenous, consume a monotonous diet, and inhabit a well-defined environment, in which the micro-biota is shared between co-housed cagemates through coprophagia. The latter observation probably accounts for the substantial ‘cage effects’ observed in microbiome studies[180]. Third, similarities between mice are amplified further in germ-free or gnotobiotic studies in which even fewer differences are present between individual animals. This uniformity contributes to a high signal-to-noise ratio and enables investigators to observe reproducible outcomes with relatively low numbers of experimental animals. Such tightly controlled experimental conditions have been critical in establishing cause–effect relationships and uncovering novel biological responses and pathways.

By contrast, humans live in a highly variable environment, exhibit genetic diversity, and consume a variable diet. This inherent variability is even more pronounced in IBD in which disease activity, medication use, and numerous environmental factors such as smoking can modulate the individual response to the gut microbiota and their metabolites. Indeed, intersubject variability is often one of the largest sources of variance in human gut microbiome studies, leading to a low signal-to-noise ratio that can obscure meaningful biological outcomes. Thus, one of the lessons learned from microbiota-based interventional studies in humans might be that such approaches show a hint of promise but will require further adjustment to amplify the favourable clinical response above the threshold of normal human variation. To address these issues, there is a need for large prospective longitudinal studies such as the Genetic Environmental Microbial (GEM) Project to define interactions

between human genetics, environmental factors, and microbial composition that contribute to the development of IBD[181].

It is important to recognize that the relationship between dysbiosis and IBD is probably complex and dynamic, rather than one of simple cause–effect. Thus, the view of dysbiosis as the response of a complex micro-bial community to the environmental stress of inflammation or medication use is not incompatible with the notion that dysbiosis plays a direct role in IBD pathogenesis. For example, dysbiosis might not be the inciting event but might develop later in the course of IBD and contribute to disease progression and chronicity. Studies of next-generation probiotics and larger controlled FMT trials could provide proof-of-concept in support of this notion.

Alternatively, it is possible that the gut microbiota have a critical role in the initiation of disease but that the window for such an effect occurs early in life. Concurrent with industrialization, the incidence of Crohn's disease and ulcerative colitis has risen globally, revealing a number of important associations between early life exposures and IBD[13]. Thus, birth by caesarean section, childhood exposure to antibiotics, use of infant formula, and residence in a sanitized environment have emerged as risk factors for the development of IBD and other immune-mediated diseases[13]. Conversely, childhood exposure to house dust in a rural setting is protective against the development of asthma, providing a cogent example of this principal[182]. Moreover, mouse models in which the gut microbiota are altered in early life phenocopy the associations with diseases observed in humans[183,184]. Although it might be difficult to demonstrate efficacy of a microbiota-based strategy for disease prevention, such an approach could be enourmously impactful, making it worth the investment in time, energy and resources.

In conclusion, we believe that association studies, animal models and early therapeutic trials collectively point to an important role of the gut microbiota and their metabolites in IBD pathogenesis. Translating these insights into viable therapeutic approaches might be challenging and require an investment in human subject research to definitively demonstrate cause–effect relation ships. Nevertheless, given the growing incidence of IBD worldwide and its association with environmental triggers, research into the gut microbiota with an eye towards therapeutics will be critically important.

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Glossary

Crohn's disease

A chronic inflammatory bowel disease that can involve the entire gastrointestinal tract and is characterized by areas of transmural inflammation surrounded by normal mucosa

Ulcerative colitis

A chronic inflammatory bowel disease characterized by diffuse mucosal inflammation limited to the colon

Dysbiosis

An alteration of the microbiota that is associated with disease

Faecal diversion

Surgical diversion of the faecal stream by means of a loop ileostomy or colostomy

Virome
The collection of prokaryotic and eukaryotic viruses that are part of the human microbiota

Bacteriophages
Viruses that infect and replicate within bacteria

Bile acids
Steroid acids found in bile that aid in fat emulsification and nutrient digestion

Short-chain fatty acids
Fatty acids with less than 6 carbons produced by bacterial fermentation of dietary carbohydrates

Immunoglobulin A
(IgA). The most abundant antibody type, mostly associated with mucosal surfaces

Ileocolonic resection
Resection of the terminal ileum, caecum and ascending colon, followed by an ileocolonic anastomosis

Ileostomy
A surgical operation in which a piece of the ileum is diverted to an artificial opening in the abdominal wall

Probiotics
Living microorganisms which, when administered in adequate amounts, might confer health benefits on the host

Enteral nutritional therapy
(ENT). Nutritional supplementation via a nasoenteric feeding tube

Faecal microbiota transplantation
(FMT). The administration of microorganisms derived from the stool of a healthy donor to the gastrointestinal tract of a patient

Coprophagia
The consumption of faeces

Box 1

Limitations of current IBD microbiome research in humans

- Wide clinical spectrum of ulcerative colitis and Crohn's disease cannot be captured in single studies
- Many microbial taxa are fastidious and difficult to culture
- Microbiome studies have focused on bacteria with relatively little known about other microorganisms, including fungi and viruses, as well as how they interact with each other
- Microbiota composition is markedly different between faecal and mucosal samples, yet most analyses of microbiome communities have been based on faecal samples Most studies focus on microbiota composition rather than function
- Most studies characterize the gut microbiota using 16S ribosomal RNA tagged sequencing rather than shotgun metagenomics with deep sequencing to provide strain-level taxonomic classifications
- Microbiome studies in IBD are confounded by treatment interventions and the effects of inflammation
- Most published results are based on cross-sectional and not prospective longitudinal cohort studies

Box 2

Limitations of current IBD mouse models

- Most mouse models rely on gene knockouts, whereashuman risk alleles seldom lead to complete loss of function
- Mouse models usually explore the effect of a single gene, whereas in humans there are often multiple alleles involved
- Immune responses differ between mice and humans
- Mice do not capture the genetic and environmental diversity of human populations
- Mouse experiments fail to account for variables such as medication exposure, smoking and diet that are inherent in human research

Box 3

Outstanding questions for the role of the gut microbiota in IBD

- What are the very early events in IBD pathogenesis? Specifically, does dysbiosis precede the development of inflammation, or does inflammation arise independently of the microbiota and lead to dysbiosis?
- Can microbiota testing be used as a reliable marker of disease onset and progression?
- What are the antigens in the intestinal lumen that drive T-cell activation in IBD?
- What is the best way to administer faecal microbiota transplantation (FMT) with regards to patient selection, donor selection, and mode of administration?
- What will be the long-term outcomes of FMT for IBD? Specifically, how durable will the effects be?
- How can we refine microbial therapies beyond FMT? Can we re-engineer the microbiota of individual patients based on their specific disease phenotype, genetic makeup, and microbiome?
- Can microbial-based therapies be used to prevent, rather than treat, IBD?
- What is the role of the virome in IBD?

Key points

- Alterations in intestinal microbial composition have long been associated with chronic inflammation; however, a definitive cause–effect relationship between dysbiosis and IBD has been difficult to prove, especially in humans
- Dysbiosis alters not only the composition of the intestinal microbiota, but also its metabolome, thereby exerting a wide range of effects on the host
- While the microbiota plays a key pathogenic role in IBD, chronic inflammation, in turn, promotes dysbiosis by altering the oxidative and metabolic environment of the gut
- Animal studies have elucidated key immunological pathways in the pathogenesis of IBD, established both pro-inflammatory and anti-inflammatory roles of the gut microbiota, and shown that the gut microbiota is indispensable for pathogenesis in most colitis models
- Microbial-based treatments will likely have a role in the future management of IBD; however, many questions remain regarding the bacterial composition, timing of administration, and patient selection for such therapies

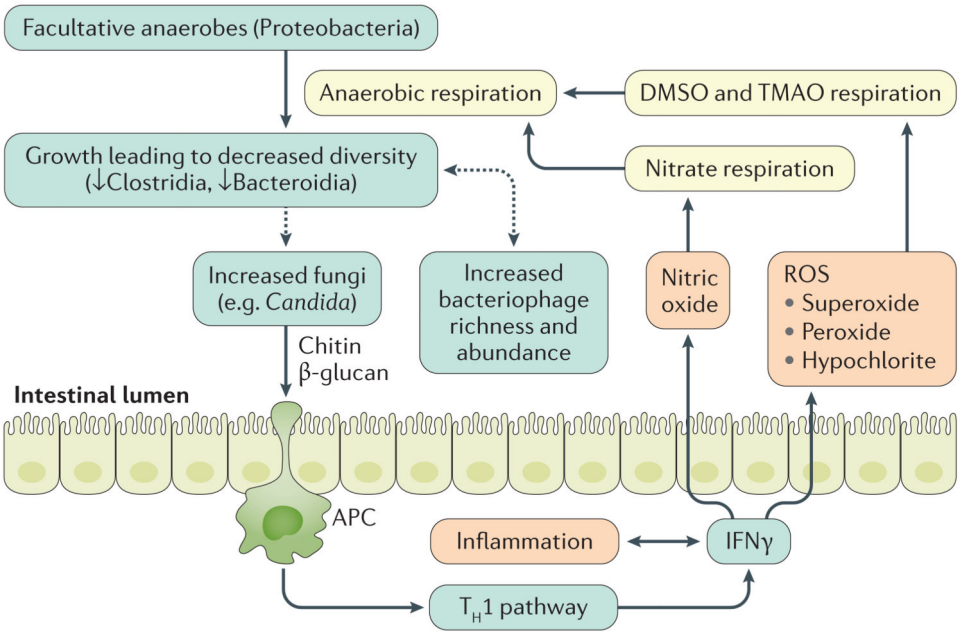

Figure 1. Colonic inflammation in IBD and link to the gut microbiota

Colonic inflammation stimulates IFNγ production, which generates reactive oxygen species (ROS) by phagocytic innate immune cells. These radicals eventually form products for anaerobic respiration. Facultative anaerobes utilize these products to outgrow, causing decreases in bacterial diversity. The dysbiotic microbiota might further encourage the outgrowth of fungi, especially *Candida,* which can in turn exacerbate inflammation via chitin and β -glucan antigen-presenting cell (APC) activation of the type 1 T helper (T_H1) pathway. Similarly, the dysbiotic microbiota are associated with increased bacteriophage richness and abundance, which can in turn modify the bacterial microbiota via gene transfer. DMSO, dimethyl sulfoxide; TMAO, trimethylamine *N*-oxide.

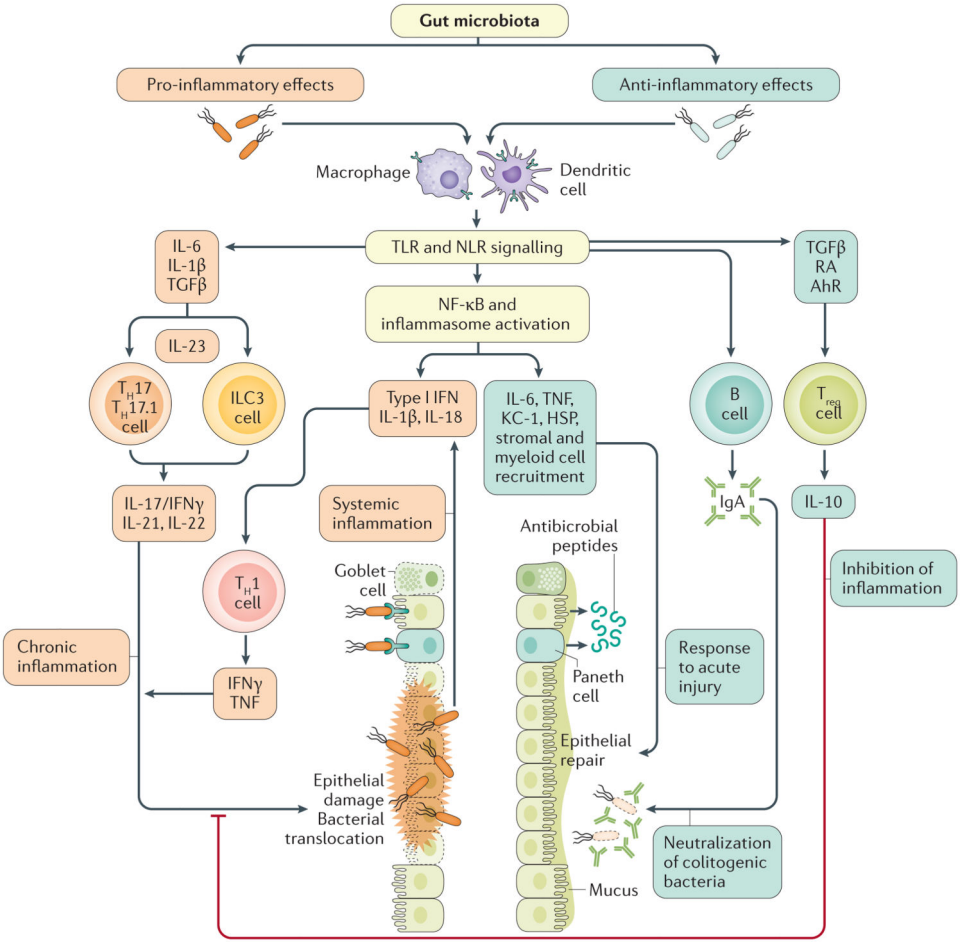

Figure 2. Pro-inflammatory and anti-inflammatory effects of the gut gut microbiota

Pathogenic microorganisms are sensed via Toll-like receptors (TLR) and NOD-like receptors (NLR) on innate immune cells (dendritic cells (DCs), macrophages), Paneth cells and epithelial cells. This process leads to differentiation of type 17 T helper (T_H17) cells and type 3 innate lymphoid cells (ILC3s) under the influence of transforming growth factor (TGF)β, IL-6 and IL-1β, and activation of the IL-23 inflammatory pathway. TLR and NLR signalling also leads to NF-κB and inflammasome activation, secretion of pro-inflammatory cytokines, and type 1 T helper (T_H1) cell activation. Ultimately, these inflammatory responses lead to epithelial damage, loss of mucus-secreting goblet cells, and bacterial translocation, which further stimulates the inflammatory response. Anti-inflammatory bacteria are also sensed via TLR and NLR; however, this process leads to regulatory T (T_{reg})-cell differentiation via TGFβ, retinoic acid (RA) and the aryl hydrocarbon receptor (AhR) signalling. T_{reg} cells exert their immunoregulatory function via IL-10 secretion. Moreover, NF-κB and inflammasome activation lead to secretion of anti-apoptotic factors and antimicrobial peptides, alongside recruitment of stromal and myeloid cells necessary for epithelial repair. Finally, IgA (produced by B cells) prevents colitogenic bacteria from penetrating the mucus layer.