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REVIEW

The microbiota in inflammatory bowel disease

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Abstract This review explores our current understanding of the complex interaction between environmental risk factors, genetic traits and the development of inflammatory bowel disease. The primacy of environmental risk factors is illustrated by the rapid increase in the incidence of the disease worldwide. We discuss how the gut microbiota is the proximate environmental risk factor for subsequent development of inflammatory bowel disease. The evolving fields of virome and mycobiome studies will further our understanding of the full potential of the gut microbiota in disease pathogenesis. Manipulating the gut microbiota is a promising therapeutic avenue.

Keywords Inflammatory bowel disease · Gut microbiota · Mycobiome · Virome · Faecal microbial transplantation

Introduction

The gut microbiota is altered in patients with inflammatory bowel disease (IBD). As the incidence of IBD rises worldwide [1] with socioeconomic development, environmental factors associated with modern life appear to be driving these microbial changes. Although genetic studies provide insight into disease mechanisms, the environmental influence on the microbiota may be the essential factor in disease development. The virome and mycobiome have been relatively neglected areas of research, but given their intricate interactions with the gut bacteria, future research in these fields will be important in understanding of the pathogenesis of IBD and its treatment.

The gut microbiota: the proximate environmental risk factor for IBD

The gut microbiota is the proximate environmental influence on the risk of IBD (Fig. 1), but it is unclear whether tissue damage results from an abnormal immune response to a normal microbiota or from a normal immune response against abnormal microbiota. The primacy of environmental risk factors in the development of IBD is demonstrated in twin studies. The concordance rate for ulcerative colitis (UC) is less than 20 % and is around 50 % for Crohn's disease (CD) in monozygotic twins [2–4]. Of note, twin studies have not provided much support for a host genetic influence on the gut microbiota [5]. Healthy siblings of patients with CD also display altered microbial and immune profiles associated with CD, distinct from their genotype-related risk [6]. These findings suggest that although genes and the environment are important in disease development, the environment has a greater effect, especially in UC.

The increasing prevalence of IBD worldwide also supports the primacy of environmental risk factors [7, 8] in the development of IBD, except in rare cases of monogenic disease [9]. The most consistent epidemiological feature of

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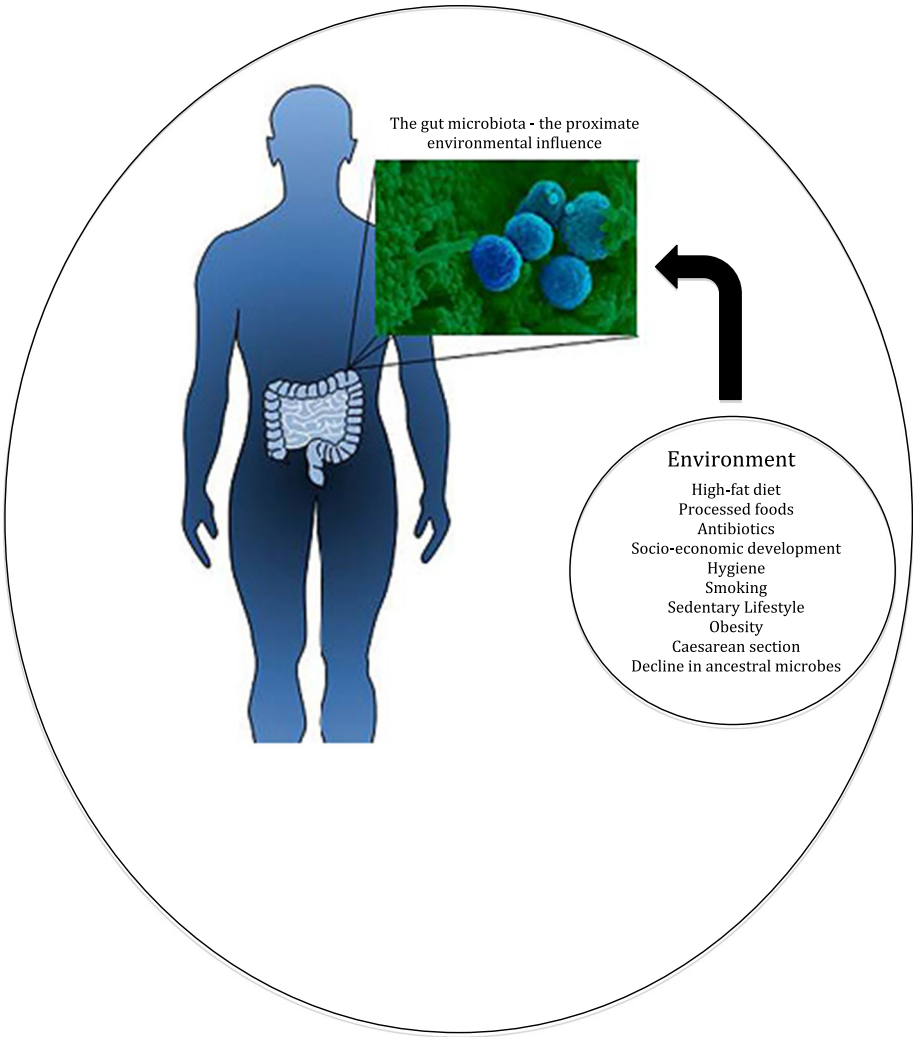

Fig. 1 The gut microbiota is the proximate environmental influence on inflammatory bowel disease

both UC and CD is the increase in incidence and prevalence when a society transitions from developing to developed [10]. The rapid pace of these epidemiological changes is too brisk for them to result from changes in gene frequency, pointing to an environmental effect on the risk of disease. Studies of emigrant groups support this hypothesis [7]. Studies of migrant populations suggest early childhood environmental influence is crucial; children take on the risk profile of their new environment, whereas their parents maintain the risk profile of their country of origin [11].

Overlapping genetic and environmental risk factors for IBD

Important environmental and lifestyle risk factors for the development of IBD include smoking [12], industrialization and socioeconomic development [13], oral contraceptive use [14] and diet [15].

Since the discovery of *NOD2* [16, 17], more that 160 IBD-associated gene loci [18] have been identified. These genetic associations highlight the importance of gene–microbe–environment interactions in IBD pathogenesis. Key IBD risk gene pathways include sensing of the microbiota, regulators of the response to microbiota and barrier function (Table 1).

Gene–environment interactions

Environmental exposures in genetically susceptible individuals are believed to be a prime driver of IBD development. Smoking is a major environmental risk factor, with evidence for gene-microbe interactions in its contribution to disease. It has been independently demonstrated that *Nod2*$^{-/-}$ mice have altered gut microbiota composition [29], and that cigarette smoke can alter *NOD2* expression and function in intestinal epithelial cells [30].The polarizing effects of smoking on CD and UC emphasize the

Table 1 Representative inflammatory bowel disease (*IBD*) gene risk loci that regulate sensing of and response to the microbiota

Genes	Comment	References
NOD2	CD-linked intracellular sensor of bacterial peptidoglycans	[16, 17]
ATG16L1, *IRGM*	CD risk autophagy genes involved in intracellular processing of bacteria	[19, 20]
IL23R, *JAK2*, *IL12B*	IL-23–T_h17 pathway linked to IBD and the autoimmune conditions psoriasis and ankylosing spondylitis	[21]
IL10	Recessive mutations linked to very early onset IBD	[22]
MUC19	Involved in mucus production and mucosal barrier function	[23]
SLC22A5, *GPR35*	Immune response to bacterial-derived ligands and metabolites	[24]
IL27	Maintenance of epithelial barrier against commensal bacteria	[25]
ECM1	Glycoprotein that interacts with the basement membrane, inhibits matrix metalloproteinase 9 and can activate NF-κB. UC risk gene	[26]
PTPN22	CD risk gene with role in autoimmunity. Protective effect in UC	[27]
IKBL	MHC gene associated with severe UC	[28]

CD Crohn's disease, *MHC* major histocompatibility complex, *NF-κB* nuclear factor κB, *T_h17* type 17 helper T cell *UC* ulcerative colitis

complexity of disease pathogenesis [31, 32]. In contrast to CD, where smoking confers an elevated risk, smoking may suppress the risk of UC in genetically predisposed individuals until cessation of smoking. Unmasking of symptoms or precipitation of onset of UC may occur with removal of the potentially protective effect of smoking [33]. Another hypothesis is that smoking may influence disease phenotype at disease onset, resulting in the development of CD rather than UC in a proportion of smokers [33]. This may, in part, explain the apparent protective effect of smoking status on UC. In an intervention study, some patients with typical CD developed UC-like lesions of the distal colon after smoking cessation [34]. Smoking cessation is associated with an increase in abundance of *Firmicutes* and *Actinobacteria* and with a decrease in abundance of *Bacteroidetes* [35]. Microbial changes induced by smoking, along with other biological changes to mucosal homeostasis, may account for the variance of smoking as a risk factor for disease.

Diet–microbe–gene interactions

Diet is a major environmental risk factor that can directly influence gut microbial composition [36]. Population changes in dietary habits towards a processed diet with high fat and high sugar content is strongly linked to socioeconomic development, which itself has a strong epidemiological association with IBD. Several studies illustrate how dietary environmental insults resulting in compositional changes to the microbiota could theoretically lead to inflammation in genetically susceptible individuals. One report suggests that dietary-fat-induced taurocholic acid promotes the growth of the pathobiont *Biophilia wadsworthia* and induces colitis in *Il10*$^{-/-}$ mice [37]. This has not been demonstrated in humans, but genetic defects in IL-10 and/or its receptor have been associated with IBD [22, 38].

Industrialized food processing and the use of food additives is a modern phenomenon. It has been shown that emulsifiers, commonly used food additives, can modulate the mouse gut microbiota, with resultant promotion of inflammation and metabolic syndrome [39]. Low concentrations of two commonly used emulsifiers, carboxymethylcellulose and polysorbate 80, induced low-grade inflammation and obesity in wild-type mice, and promoted colitis in genetically predisposed *Il10*$^{-/-}$ and *Tlr5*$^{-/-}$ mice.

Gene–gene interactions

Many of the genetic loci that confer risk in IBD interact. The severity of disease phenotype may be determined by gene–gene interactions in the context of gene–microbe interactions. The direct interaction between NOD2 and autophagy genes is one example of this [40]. Activation of *NOD2* by bacteria and bacterial ligands results in *ATG16L1*-mediated formation of autophagic vacuoles in epithelial and dendritic cells [41]. This microbial-stimulated autophagy induction is impaired in both *NOD2* and *ATG16L1* variants associated with CD. It appears that normal *NOD2* function is necessary for recruitment of *ATG16L1* to the plasma membrane at the site of bacterial entry and thus for the wrapping of invading bacteria by autophagosomes [40]. *NOD2*-dependent defects in bacterially induced autophagy responses have been demonstrated for adherent–invasive *Escherichia coli* bacteria, which are strongly associated with ileal CD, and *Salmonella typhius* and *Shigella*, which are also linked to CD.

Another example of gene–gene interactions is the endoplasmic-reticulum-stress-induced unfolded protein

pathway. The unfolded protein pathway genes *XBP1* and *ORMDL3* have been associated with CD and UC, and are closely linked to autophagy [42]. Autophagy appears to be a key converging pathway for the strongest genetic risk factors for IBD that result in inappropriate innate immune responses to the microbiota.

Who becomes sick and the timing of onset

The requirement for genes, microbes, a host and the environment in determining who becomes sick was elegantly shown in a study in which genetically susceptible mice became sick only in the presence of commensal bacteria, intact gut immunity and exposure to an environmental virus [43]. The co-occurrence of risk factors determines who becomes sick, but infections may determine the timing of disease onset. Disruption of the mucosal barrier by infectious or other environmental agents exposes the host immune system to the resident microbiota, leading to proliferation of pathogen-specific and commensal-specific T cells [44]. These cells migrate to other mucosal sites, where they react with the commensal microbiota and can tip the balance from physiologic to pathologic inflammation. As the common mucosal immune system enables lymphocytes to migrate among different mucosal tissues, it is possible that commensal-specific T cells generated by infection at an extraintestinal site might migrate to the intestine. This might account for some patients developing relapses of IBD with concurrent infections.

The changing phenotype of IBD

The phenotype of patients with IBD has changed in recent decades (Table 2), and the microbiota may have contributed to this. There have been changes in disease behaviour, risk of surgery and nutritional status. There have also been changes in the incidence of IBD amongst different ethnic groups as they are exposed to increasingly industrialized environments [8].

Disease phenotype at diagnosis of IBD has changed over recent decades. A Danish study investigating consecutive population-based cohorts describes these changes. The proportion of CD patients amongst the total IBD cohort increased and the prevalence of CD and UC patients who were smokers at diagnosis decreased with time. The median age at diagnosis was stable over five decades for CD patients, but increased from 34 to 38 years in patients with UC [45].

A Dutch population study of patients with newly diagnosed IBD in 2006 found 61 % of patients with CD had ileal involvement, 31 % of patients had stricturing or penetrating disease and in 4 % of patients there was upper gastrointestinal tract involvement at diagnosis [54]. For CD patients, the mean age at diagnosis was 36.7 years. In the Olmsted County cohort study (1970–2004), 81.4 % of patients had non-stricturing, non-penetrating disease at diagnosis; 64 % had ileal involvement at diagnosis [55]. The phenotype at diagnosis in patients with UC is generally split equally among proctitis, left-sided disease and pancolitis [56, 57]. The proportion of patients presenting with pancolitis increased over the five decades in Denmark [45]. IBD in patients with primary sclerosing cholangitis is a distinct phenotype, with such patients having increased risk of pouchitis (not related to the severity of liver disease) [58] and colorectal cancer, in addition to risks of cholangiocarcinoma, liver failure and gallbladder cancer.

The phenotype of disease amongst Asian patients with IBD can differ from that of patients from western Europe and North America [59]. Male predominance [57] of increased ileocolonic disease has been described amongst Asian cohorts of patients with CD. However, a recent study failed to show a significant difference in disease location between Asian and Australian cohorts [57].

Twin studies have shown that phenotype is an important factor in determining changes in gut microbiota [5]. Ileal CD patients were found to have lower abundance of *Firmicutes* compared with healthy controls, whereas colonic CD patients had increased abundance [5]. Conversely, the abundance of *Fusobacteria* was increased in ileal CD patients and decreased in colonic CD patients compared with healthy controls [5]. An increase in abundance of *Proteobacteria* was reported in ileal CD patients compared with healthy controls, but this finding was not observed between healthy controls and colonic CD patients [5]. Disease phenotype appears to outweigh the effects of genotype on the microbiome, although studies in twins do not differentiate early environmental influences on the microbiota that persist from genetic influences [60]. A large study of paediatric patients with treatment-naïve IBD found the presence of deep ulcers on endoscopy was associated with increased abundance of *Pasteurellacaea*, *Veillonellacea* and *Rothia mucilaginosa* [61]. Disease location (colonic, ileocolonic and ileal) did not significantly disrupt the similarity between rectal- and ileal-biopsy-associated microbiome profiles [61].

Obesity has reached epidemic proportions in Western countries, becoming an equal if not greater contributor to burden of disease than smoking in the USA [62]. Regression in life expectancy in the twenty-first century is predicted if the rate of obesity remains unchecked [63]. Malnutrition has long been recognized as a complication of IBD. Previously, attention focused on patients who were underweight, but obesity is increasingly associated with IBD [47].

Table 2 The changing phenotype of patients with inflammatory bowel disease (*IBD*)

Feature	Comment	References
Increased BMI	Prevalence of obese and overweight patients in a Scottish IBD population was 18 and 38 %, respectively	[46]
	17 % of patients with CD in an Irish cohort were obese compared with 12 % of controls	[47]
	Increased weight of patients with CD enrolling in clinical trials (1991–2008)	[48]
	23 % of paediatric patients with IBD in the USA were found to be overweight or obese	[49]
Decreased rate of surgery	Cumulative probability of first major surgery at 9 years decreased from 50 % (1979–1986) to 23 % (2003–2011) in patients with CD, and from 14 to 9 % in patients with UC	[50]
	Decreased risk of surgery in patients in whom CD was diagnosed after 1996, associated with increased specialist care	[51]
Increasing prevalence of elderly-onset IBD	Increased proportion of colonic disease and inflammatory behaviour in elderly patients with CD	[52, 53]
	Progression of disease behaviour less than in younger patients. Milder disease course	[52]

BMI body mass index, *CD* Crohn's disease, *UC* ulcerative colitis

Data from a northern European population reported that the prevalence of obese and overweight patients in an IBD population was 18 and 38 %, respectively [46]. In the overweight/obese cohort of UC patients there were higher levels of surgery, but the converse was true for the CD cohort. In that study there were significantly more obese patients with CD than with UC [46]. There has been an increase in weight and disease activity in patients with CD enrolling in clinical trials in the last 20 years [48].

A large prospective study found no association between obesity and development of incident IBD [64]. This cohort had a predominance of middle-aged subjects, the median age being approximately 53 years. IBD tends to occur at an earlier age. Conversely, a recent case–control study investigated a cohort of patients aged 50–70 years, finding obesity was commoner in patients with CD than in community controls without IBD [65]. A subsequent study of American women found that obese women were at increased risk of developing CD [66].

Earlier paediatric IBD populations have been described as being underweight and malnourished, with lower body mass index (BMI) than the normal distribution [67]. However, studies since the turn of the millennium have revealed that children with IBD are affected by current population trends towards weight gain; 10 % and 20–30 % of incident CD and UC patients were overweight or at risk of being overweight as per BMI [68]. These studies also showed that 22–24 % and 7–9 % of incident CD and UC patients had low BMI.

A large, multicentre cohort study of children (1,598) with IBD was performed in the USA, where childhood obesity has become epidemic; 31.7 % of children are estimated to be overweight or obese [69]. The overall prevalence of overweight or obesity in this IBD population was 23.4 %, with 20 and 30.1 % of the CD and UC population overweight or obese, respectively [49]. Paediatric patients with CD who are overweight or obese were found to have higher rates of IBD-related surgery, which is similar to findings in adult populations [70]. Corticosteroid use was associated with the overweight/obese UC group (35 % vs 27 %) but not the CD group.

The rise of obesity is especially concerning in patients with IBD as it a known risk factor for colorectal cancer (CRC) [71], and can affect the efficacy of medical treatment [72]. We have previously reviewed the role of the gut microbiota in obesity [73]. Similarly to IBD, obesity is an increasing health issue worldwide, with dietary, environmental and genetic factors important in the development of obesity. Early exposure to antibiotics in mice alters their microbiota, leading to lasting effects on body composition [74]. Exposure to antibiotics in the first years of life is associated with early-childhood obesity [75]. Onset of IBD during childhood is also associated with antibiotic use in the first year of life [76]. A metagenomic systems biology computational framework identified both network-level and gene-level topological differences associated with IBD and obesity [77]. These "Western" diseases are increasing in prevalence, and manipulating the gut microbiota is an attractive therapeutic approach in their management. Strategies for combating obesity and related conditions that involve manipulating the gut microbiota include bariatric surgery [78, 79], microbial transplantation [80, 81] and probiotics [82].

Microbiota studies in patients with IBD

Studies profiling the gut microbiota in patients with IBD compared with controls have consistently shown changes in microbiota composition as well as reduction in overall biodiversity [83] (Fig. 2, Table 3). The largest study to date in a treatment-naïve cohort of paediatric patients with CD [61], in whom analysis of mucusol and lumen-associated microbiota was performed, confirms that inflammation

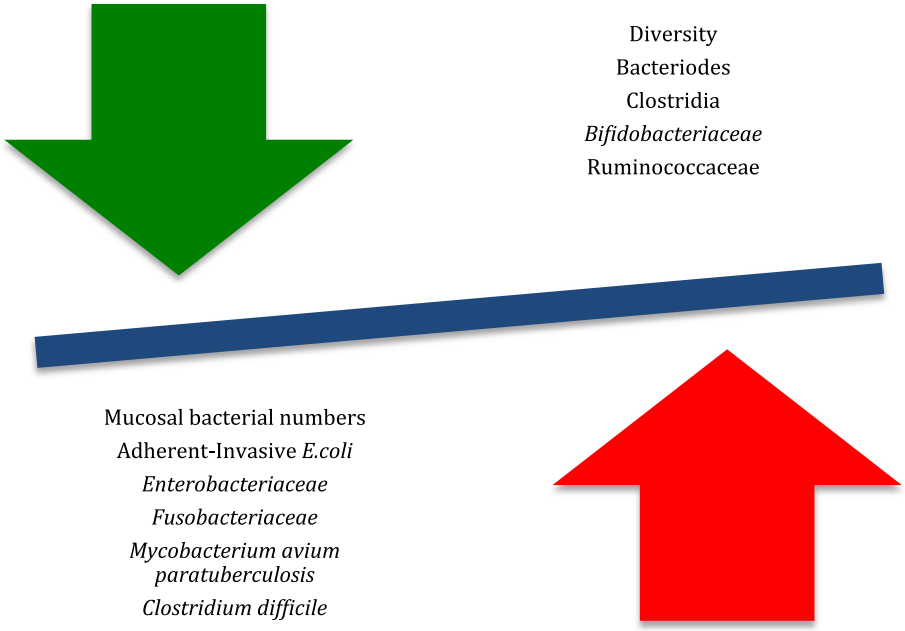

Fig. 2 Major findings from microbial studies of patients with inflammatory bowel disease

Table 3 Microbiota in inflammatory bowel disease (*IBD*)

Bacteria	Comment	References
Adherent–invasive *Escherichia coli*	Abundance increased in ileal CD	[84]
Fusobacterium	Associated with CD, UC and CRC	[61, 93, 94]
Faecalibacterum prausnitizi	Abundance of this butyrate-producing member of the family *Ruminococcaceae* reduced in ileal CD	[101]
Roseburia	Clade XIVa *Clostridia*, associated with anti-inflammatory T cell production. Abundance reduced in CD and UC	[88]
Odoribacter	Phylum *Bacteriodes*. SCFA producer. Abundance reduced in pancolonic UC and ileal CD	[88]
Bifidobacterium	Abundance decreased in CD	[61]
Anaerostipes	Phylum *Firmucutes*. Butyrate producer. Abundance decreased in current or former smokers	[88]
Mycobacterium avium subsp. *paratuberculosis.*	Linked to CD	[105]
Clostridium difficile	IBD patients have increased risk of colonization and infection	[106]

CD Crohn's disease, *CRC* colorectal cancer, *SCFA* short-chain fatty acid, *UC* ulcerative colitis

is strongly associated with an overall drop in species diversity and alterations in the abundance of several taxa. The disease state was associated with increased abundance of *Enterobacteriaceae*, *Fusobacteriaceae*, *Pasteurellaceae*, and *Bifidobacteriaceae*.Significantly, the imbalance in microbiota compostion described was observed only in the analysis performed on tissue samples; much weaker associations were described in stool sample analysis, suggesting less dramatic shifts in the luminal microbiota despite a disease state [61]. Microbiota profiles at diagnosis predicted follow-up clinical outcomes as measured by the paediatric CD activity index: the levels of *Enterobacteriaceae* were negatively correlated with future paediatric CD activity index and the levels of *Fusobacterium* and *Haemophlius* were positively correlated.

Significant research interest has focused on the role of *E. coli* particularly in patients with ileal CD [84], with studies consistently demonstrating increased levels of mucosa-associated *E. coli*. A new pathogen strain with adherent and invasive behaviours termed "adherent-invasive *E. coli*" has been isolated from ileal biopsy samples of patients with CD [85]. Adherent-invasive *E. coli* bacteria have the ability to invade epithelial cells and replicate within macrophages [86]. Although they can be present in healthy individuals, it has been shown that they are unable to adhere to ileal enterocytes isolated from healthy

individuals [87]. This suggests the inflamed ileum provides a niche environment for these pathobionts, which may influence disease exacerbations and chronicity. The genera *Escherichia* and *Shigella* have been found to be highly enriched in ileal CD [88]. *E. coli* bacteria produce lipopolysaccharide, which can trigger the inflammatory cascade via Toll-like receptor 4 signalling [89]. Mutations in the *TLR4* gene are associated with CD and UC [90], and *TLR4* expression is upregulated in intestinal epithelium of IBD patients [91]. It has also been demostrated that mesalamine use is linked to a strong reduction in the abundance of *Echerichia* and *Shigella* [88, 92].

Increased abundance of *Fusobacteria*, another phylum of adherent and invasive bacteria, has been associated with both UC and CD [93]. *Fusubacterium* species have also been linked to CRC [94]. They are enriched in tumour tissue compared with adjacent normal colon in patients with CRC, in adenomas compared with normal tissue, and in stool samples from patients with both adenomas and adenocarcinomas [95]. Possible mechanisms of action in stimulation of growth of CRC relate to the ability of *Fusobacterium* to invade and induce oncogenic and inflammatory responses through its unique FadA adhesin, which binds to E-cadherin and activates β-catenin signalling [96]. Potential mechanisms of function of *Fusobacterium* in IBD have not been described, but the invasive ability of *Fusobacterium* has been positively correlated with the IBD status of the host [97]. *Fusobacterium* provides a potential theoretical link given the increased risk of CRC associated with IBD.

The depletion of certain bacteria and loss of their protective functions are likely to have a significant impact on disease course. Many of the protective functions of the bacteria linked to IBD relate to their ability to ferment dietary fibre to produce short-chain fatty acids (SCFA) [98]. SFCA are a key source of energy for colonic epithelial cells [99], and regulate colonic regulatory T cell homeostasis [100]. In ileal CD, the abundance of members of the family *Ruminococcaceae*, in particular *Faecalibacterium*, is reduced [88, 101]. *Faecalibacterium prausnitizi* is a major producer of the SCFA butyrate, exhibits anti-inflammatory effects in a colitis setting [102] and provides the first step in microbiome-linked carbohydrate metabolism by degrading dietary polysaccharides [103]. Reductions in the abundance of *F. prausnitizi* have been associated with higher risk of postoperative recurrence of ileal CD [102], and administration of *F. prausnitizi* reduces inflammation in mouse models [102]. *Roseburia*, the abundance of which is reduced in all IBD subgroups [88], is connected to the family *Ruminococcaceae* as it relies on its members to produce acetate, which it uses to produce butyrate [104]. The abundance of *Odoribacter splanchus*, another SCFA producer, is reduced in patients with pancolitis and in patients with ileal CD [88]. All the above-described changes in bacterial populations have plausible functional consequences for the ability of the host to regulate inflammation mediated in part by the effect on SCFA production.

Metagenomic studies have identified functional microbiome pertubations in IBD [88]. Metagenomic analysis is important as the functional composition of the microbiota exhibits more stability than the microbiota at the phylogenetic level over time and between individuals [83]. One study identified only nine bacterial clades associated with UC patients compared with controls, but 21 differences in functional and metabolic pathways, with similar findings for CD patients [88]. Changes seen in both UC and CD include decreased expression of genes involved in amino acid metabolism and synthesis, and increased expression of genes related to sulfate transport as well as genes involved in glutathione transport. Glutathione is significant as it is produced by *Proteobacteria* and enteroccocci, and is involved in the maintenance of bacterial homeostasis during oxidative stress. Ileal CD is associated with specific alterations in microbiota gene expression, including increase in expression of genes involved in glycolysis and carbohydrate metabolism and reduction in expression of genes involved in lipid metabolism, indicating alterations in energy metabolism. Increased expression of adherance–invasion and type 2 secretion system genes that are involved in pathologic processes typical of pathobionts is also seen. Expression of these genes results in tissue destruction and forms part of a cycle of inflammation [88].

The gut virome and IBD

Human microbiota studies to date have focused on bacteria, generally neglecting the non-bacterial components of the gut microbiota. Viruses, which include phages, in fact compromise the most abundant biological entities within the gut, greatly outnumbering bacteria, with an estimated total of 10^{15} [107]. The function of viruses in the healthy gut and in gastrointestinal disease is not well defined; however, a number of studies suggest an important role. It has been demonstrated that a common enteric RNA virus can replace the beneficial function of commensal bacteria in the intestine [108]. In that study, infection of germ-free or antibiotic-treated mice with murine norovirus (MNV) restored intestinal morphology and lymphocyte function without inducing overt inflammation. MNV infection was also shown to offset the deleterious effect of treatment with antibiotics in models of intestinal injury and pathogenic bacterial infection. This study supports the hypothesis that similarly to bacteria, eukaryotic viruses have the capacity to support intestinal homeostasis and shape mucosal

immunity. As mentioned in mouse models of the *Atg16L1* gene, a CD susceptibility gene involved in autophagy, hypomorphic *ATG16L1* disrupts Paneth cell function [109]; intriguingly, this is dependant on the environmental contribution of MNV infection [43].

Given their biological characteristics, in particular their influence on bacterial populations, there is emerging interest in the potential role of phages in IBD. Phages are bacterial viruses that can attack and kill a target bacterium [110]. They bind to specific targets on the bacterial cell surface, so individual phages generally target a very narrow range of strains of the same bacterial species. Phages shape microbial population structure [111] and maintain microbial diversity within the gut by restricting the clonal expansion of microbes that respond most efficiently to external stimuli. They do this by reacting in a 'kill the winner' dynamic referred to as the constant diversity model [112]. Several studies have already looked at phages in IBD. In mucosa biopsy samples, phage numbers were significantly higher in patients with CD than in controls [113]. Phage populations are more diverse in patients with CD than in healthy individuals, and there is interindividual variation in phage diversity in patients with CD [114]. The possibility of using phages as viral biomarkers has potential. One study found among the viral biomarkers identified, 5 % were more represented in CD patients than in healthy controls [115]. A recent publication confirms increased richness and biodiversity of phage populations in IBD patients compared with controls [116]. Specifically, increases in the abundance of *Claudovirales* in IBD patients were seen. Disease-specific changes to the virome in CD and UC were also described. The increased biodiversity of phage populations was in contrast to the expected reduced bacterial biodiversity, again described in IBD patients. Phage populations also respond to environmental factors, such as diet [117] and antibiotic exposure [118], known IBD risk factors.

The mycobiome and IBD

The mycobiome is another relatively unexplored area of research [119]. The fungal components of the gastrointestinal tract have been characterized in healthy individuals [120]. The gastrointestinal tract was found to contain *Aspergillus*, *Cryptococcus*, *Penicillium*, *Pneumocystis* and *Saccharomycetaceae* yeasts (*Candida* and *Saccharomyces*). Fungal abundance has also been correlated with consumption of a diet rich in carbohydrates [121]. *Candida* was positively correlated with carbohydrate consumption and negatively correlated with total saturated fatty acid levels. *Aspergillus* was negatively correlated with SCFA levels in people ingesting a carbohydrate-rich diet. In addition, significant correlations between fungal and bacterial taxa have been described. Interactions between fungi and bacteria in the gastrointestinal tract, as well as with dietary components, are potentially relevant to IBD. A study in IBD patients revealed higher fungal diversity in patients with CD in comparison with controls, and no disease-specific fungal species were found in the CD and UC groups [122].

Therapeutic manipulation

Current approaches used in the therapeutic manipulation of the gut microbiota include antibiotics, probiotics and prebiotics or combinations thereof (synbiotics). Faecal microbiota transplantation (FMT) has garnered much interest as a treatment for IBD, especially UC.

Antibiotics may have a role in inducing remission in active disease and preventing relapse in some patients with IBD. Their use though is generally restricted to colonic disease [123] and complications of CD [123]. Metronidazole, in combination with azathioprine, has also shown efficacy in reducing postoperative occurrence of CD [124]. A meta-analysis of antibiotic therapy in IBD found that antibiotics significantly improved outcomes [125]. However, this analysis included patients who were treated with a diverse number of antibiotics. There was also heterogeneity amongst the trials studied, making interpretation difficult. Further clinical trials will enhance our understanding of the clinical situations whereby antibiotics may be effective, and hopefully these trials will study changes that occur in the gut microbiota.

When considering using probiotics in clinical practice, one must understand that all probiotics are not equal, and their effectiveness depends on the strain, dosage and clinical condition that is the target for therapy [126]. The use of probiotics has been reviewed extensively elsewhere, but it should be noted that as IBD is a heterogeneous condition, one would expect different probiotics to be effective for different manifestations of the disease.

Faecal microbiota transplantation

FMT is a highly successful treatment for severe and recurrent *Clostridium difficile* infection, prompting further interest in its potential in IBD [127]. Several studies have been published on the use of FMT in IBD [128]. In one study, FMT was administered via enema in a small group of children and young adults [129], and no serious adverse events were reported. A further study, in which five patients with moderate to severe active UC received FMT via nasojejenul tube and enema [130], found that FMT elicited

fever and transient rises in the level of C-reactive protein. One patient in this study had a positive clinical response after 12 weeks. These studies provide limited information regarding the potential clinical benefit of FMT. Clearly, further studies are needed; several studies are registered at ClinicalTrials.gov, and the results of these are awaited.

Although FMT proved safe [131], it does give rise to novel safety concerns. Transmission of known pathogens should be avoidable with appropriate screening, but questions remain regarding the risk posed by undiscovered pathogens. The potential for inducing bacterial translocation and sepsis particularly in patients with defective mucosal barrier function is also a major concern. More theoretical is the risk of transferring an undesirable phenotype, given the numerous gut microbial associations with diseases, including colon cancer [94]. Experimental models and human studies [81] have shown that immunologic, physiologic and metabolic phenotypes can be transferred by FMT [126]. The potential for a negative phenotype transfer is particularly relevant to IBD, where recipients of FMT are likely to be young, with an added risk of carcinogenesis. Optimal donor selection is a key safety priority; donor selection needs to be refined, with greater attention on phenotype and composition and diversity of donor microbiota.

Enthusiasm for FMT in IBD is tempered by unique challenges that also offer opportunities for discovery. Host–microbe interactions in IBD are more complex than in *C. difficile*-associated diarrhoea or *Helicobacter*-related peptic ulcer disease. Thus, the traditional 'one microbe–one disease' model makes way in IBD for the idea that groups of commensals may become pathogenic in certain contexts depending on host susceptibility. The role of the microbiota in IBD may vary at different phases in the evolution of the disease. The optimal time for microbial manipulation may be in early life, when immune development is occurring in concert with gut colonization. Patients with established IBD may, in fact, have missed the window of opportunity [132]. UC and CD are heterogeneous disorders, suggesting that microbial-based strategies may require the identification of responder subgroups. This highlights the need for microbial and other biomarkers of disease subsets. Further unknowns surround the optimum composition of the donor stool and whether it should be matched to the recipient's genotype and phenotype.

Faecal biotherapy for IBD is currently a discipline in its infancy, and future developments are likely to see the use of more sophisticated and targeted approaches that use defined microbial ecosystems with precise mixtures of the minimal microbes from stool needed to achieve specific benefits [132]. Such an approach has already been successfully used in the RePOOPulate study [133], where a selected microbial ecosystem of 33 bacteria from a single donor was used to treat *C. difficile* infection in two patients. Technologies using minimal microbiota mixtures have considerable potential advantages, such as avoiding the need for repeated stool donation, reducing the chance of the transfer of infection and allowing uniformity in the treatment given to patients. They also mark the move towards the development of a new class of therapeutics derived from the gut microbiota, with mechanism-based synthetic microbiota communities being designed to target disease-specific microbial compositional and functional imbalances.

Conclusion

If the last decade was the decade for genetics in IBD, the present is the decade of the microbiome. The next decade will focus on gene–microbe interactions and translation to the clinic.

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