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Irritable bowel syndrome, inflammatory bowel disease and the microbiome

Giles Major and Robin Spiller

Purpose of review
The review aims to update the reader on current developments in our understanding of how the gut microbiota impact on inflammatory bowel disease and the irritable bowel syndrome. It will also consider current efforts to modulate the microbiota for therapeutic effect.

Recent findings
Gene polymorphisms associated with inflammatory bowel disease increasingly suggest that interaction with the microbiota drives pathogenesis. This may be through modulation of the immune response, mucosal permeability or the products of microbial metabolism. Similar findings in irritable bowel syndrome have reinforced the role of gut-specific factors in this ‘functional’ disorder. Metagenomic analysis has identified alterations in pathways and interactions with the ecosystem of the microbiome that may not be recognized by taxonomic description alone, particularly in carbohydrate metabolism. Treatments targeted at the microbial stimulus with antibiotics, probiotics or prebiotics have all progressed in the past year. Studies on the long-term effects of treatment on the microbiome suggest that dietary intervention may be needed for prolonged efficacy.

Summary
The microbiome represents ‘the other genome’, and to appreciate its role in health and disease will be as challenging as with our own genome. Intestinal diseases occur at the front line of our interaction with the microbiome and their future treatment will be shaped as we unravel our relationship with it.

Keywords
carbohydrates, inflammatory bowel disease, irritable bowel syndrome, metagenomics, microbiota

INTRODUCTION
Inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) both present with diarrhoea, abdominal pain, accelerated transit and altered diet. They share a number of common causative features including genetic predispositions, impaired gut barrier function and altered microbiota, which will be the focus of this review.

In the past 10 years, the revolution in the speed and cost of gene sequencing has made it possible to enumerate and quantify the human microbiota: bacteria and other organisms that live in and around the human body over half of which were previously unculturable and hence unknown. The vast majority of these, estimated at 10^{14} organisms, live in the colon, and in this review will be referred to as the microbiota.

INFLAMMATORY BOWEL DISEASE
Genetic associations have proliferated in the past few years and a recent meta-analysis of genome-wide association studies reported 163 susceptibility loci, including 71 new associations [1**]. That the majority of these loci, such as NOD2, IRGM, ATG16L1 and IL23R, contribute to pathways for detecting, responding to and processing of microbiota, has emphasized the likely importance of host–microbial interactions in causing disease. Of note, Iliev et al. [2**] have recently reported a single-nucleotide polymorphism (SNP) in the CLEC7A gene associated with treatment-resistant ulcerative colitis. This gene codes for dectin-1, a C-type lectin receptor that recognizes commensal gut fungi [2**]. Mice with a CLEC7A gene deletion compared to wild
Changes in the intestinal microbiota have been found in both IBD and IBS.

Proliferation of the phylum Proteobacteria, with reduction in Firmicutes, is observed in intestinal inflammation.

Diet, particularly fat and indigestible carbohydrates, has been shown to influence microbiota and gut health, but how this translates into symptom experience is unclear.

Antibiotics and faecal transplantation are potential therapies for intestinal inflammation and functional symptoms, but the durability of this effect in the context of a potentially pathogenic diet is uncertain.

Gastrointestinal hormones

KEY POINTS

- Changes in the intestinal microbiota have been found in both IBD and IBS.
- Proliferation of the phylum Proteobacteria, with reduction in Firmicutes, is observed in intestinal inflammation.
- Diet, particularly fat and indigestible carbohydrates, has been shown to influence microbiota and gut health, but how this translates into symptom experience is unclear.
- Antibiotics and faecal transplantation are potential therapies for intestinal inflammation and functional symptoms, but the durability of this effect in the context of a potentially pathogenic diet is uncertain.

Type developed more severe dextran sodium sulphate (DSS) colitis, with increased tissue invasion, an effect prevented by the antifungal fluconazole.

The earlier findings that lower levels of Faecalibacterium prausnitzii on the ileal mucosa of patients with Crohn’s disease was associated with recurrent disease, and that F. prausnitzii could induce interleukin (IL)-10 in vitro with reduced inflammation in mouse models of colitis [3] introduced the concept that specific elements of the microbiota might modify inflammation.

Recent studies have confirmed lower levels of F. prausnitzii in adults with both Crohn’s disease [4*] and ulcerative colitis [5], but this is not essential and may be just a marker of an abnormal microbiota. A recent study which looked at new cases of paediatric Crohn’s disease actually found increased F. prausnitzii in mucosal biopsies associated with reduced bacterial diversity [6*]. However, relatives of patients with ulcerative colitis without disease also have reduced F. prausnitzii [5], suggesting that this might relate to genetic factors which in animals clearly influence the microbiota (for review, see [7**]). That this is also true in humans is supported by Li et al. [8*] who demonstrated lower levels of Clostridium group IV in biopsies of resected ileum from ileal Crohn’s disease patients without the NOD2 risk allele though interpretation is difficult owing to confounding by treatments including perioperative antibiotics. Clearer results can be seen in mouse models of ileitis (Toxoplasma gondii infection and indomethacin) in which inflammation leads to marked loss in diversity in ileal mucosal biopsies, with reduction in Firmicutes, replaced by Proteobacteria, including the adherent and invasive Escherichia coli (AIEC) [9**]. NOD2 deletion was associated with reduced numbers of Firmicutes in uninfectected mice, and after infection a dramatic shift to 99.8% Proteobacteria and loss of diversity, possibly by impairing antibacterial defences. These effects were somewhat reduced by anti-tumour necrosis factor (TNF) antibody therapy. Smoking, the environmental factor most associated with Crohn’s disease, has also been linked to a higher proportions of Bacteroidetes–Prevotella in faeces [4*]. Oral microbiota in Crohn’s disease patients, but not ulcerative colitis patients, showed reduced diversity with reduced Firmicutes and Fusobacteria and an increase in Bacteroidetes [10*]. A recent paediatric study was able to discriminate patients with IBD from other diagnoses on the basis of microbiota testing alone [11**].

Morgan et al. [12**] inferred the microbiome from the microbiota in a sample of 121 Crohn’s disease and 75 ulcerative colitis patients, and 27 healthy controls. Ileal Crohn’s disease showed the greatest difference from controls with 12% of the metagenomic pathways analysed significantly affected. IBD patients had lower levels of Ruminococcaceae and Roseburia, metabolically interlinked clades both known to produce short-chain fatty acids (SCFAs) (acetate from Ruminococcaceae utilized by Roseburia to produce butyrate), whereas Faecalibacterium, a major butyrate producer, was again reduced in ileal Crohn’s disease, compared to an abundance of Escherichia. 5-Aminosalicylate use was associated with reductions in Escherichia, whereas antibiotic use, greatest in ulcerative colitis, was associated with greatly reduced diversity. Metagenomic profiles showed that IBD microbiomes, particularly in ileal Crohn’s disease, had reduced capacity for amino acid biosynthesis and carbohydrate metabolism, with increased presence of enzymes to metabolize mucin, and transport carbohydrate and sulphate. Erickson et al. [13*] used a multi‘omics’ approach in six twin pairs from the Swedish twin cohort, both concordant and discordant for disease. They confirmed reduced levels of Faecalibacterium, as well as reduced richness of the microbiota in Crohn’s disease, most markedly with ileal disease. Analysis of the proteome showed reduced abundance of genes for CAZymes, enzymes capable of degrading complex carbohydrates typical of plant cell walls, though since dietary data were unavailable, it is not clear whether this is due to patient selection of a low fibre diet in an attempt to minimize symptoms [13*]. The increased presence, however, of Omp proteins, found on gram-negative bacteria, does suggest a shift to a microbiota more able to elicit a pro-inflammatory response through Toll-like receptor (TLR)-4 signalling.

IRRITABLE BOWEL SYNDROME

Some of the most convincing observational data for an interaction between the microbiome and IBS...
IBS, IBD, and the microbiome

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symptoms comes from studying *Campylobacter* enteritis which markedly increases gut permeability and leads to IBS in around 10%. Swan et al. [14] looked at mucosal gene expression both 6 months after *C. jejuni* infection and in IBS and identified several common alterations compared to healthy controls, including increased TNFSF15 mRNA. They went on to examine candidate genes whose expression was shown to be abnormal in both patient groups and confirmed a recent report [15] of an increase in the major allele of a TNFSF15 SNP associated with diarrhoea-predominant IBS, and also identified a TNF-α polymorphism associated with postinfectious IBS. A HapMap showed that the TNFSF15 SNPs in the two studies are closely linked so they can be regarded as replications. A meta-analysis of studies preceding this report found an increased incidence of polymorphisms associated with lower levels of IL-10 in IBS, though the small numbers even in the meta-analysis mean these studies need replication [16].

Another similarity between IBS and IBD is the association of both with increased mucosal permeability. IL-22 is recognized to regulate mucosal permeability and its secretion is modulated by IL-23, a recognized risk factor for IBD. IL-22-deficient mice have recently been shown to have altered commensal microbiota with an increased presence of Proteobacteria, and reduced Firmicutes [17**]. They developed more severe colitis in a noninfectious model, and were able to transmit this exaggerated response to co-housed wild-type mice, whose microbiota also included the Proteobacteria seen in the knockouts. IL-22 dysfunction has also been linked to increases in circulating antibodies to enteric pathogens in IBD [18*]. Several studies show IBS is associated with increased permeability (for review, see [19]). Martinez et al. [20**,21**] examined the underlying mechanism showing structural defects by electron microscopy along with down-regulation of the tight junction protein ZO1 at both gene and protein level in jejunal biopsies from IBS patients with diarrhea. Abnormalities of faecal microbiota in IBS reported in several series are inconsistent probably because confounders such as diet, smoking, age and antibiotic use have not been adequately controlled for. Better controlled intervention studies are needed to overcome this defect, so the report that faecal microbiota transplant from IBS patients into gnotobiotic mice can induce visceral hypersensitivity is a step in the right direction [22**]. The authors transplanted the microbiota of three IBS patients with constipation (IBS-C) and two healthy controls into germ-free mice. They showed a reduction in bifidobacteria and increase in *Enterobacteriaceae* and sulphate-reducing bacteria with an increase in caecal sulphide levels and hydrogen production with IBS microbiota compared to controls. This profile was similar to these authors’ previous report in 14 IBS-C patients and 12 healthy controls using selective culture techniques to enumerate bacteria divided into anaerobes, hydrolytic (using a range of substrates including cellulose, xylan, spinach, wheat), H₂-consuming and lactate-utilizing bacteria [23]. They showed no difference in hydrolytic bacteria, but a decrease in lactate-utilizing bacteria (mainly *Bifidobacteria* and *Lactobacilli*), and 100-fold increase in sulphate-reducing bacteria. Ex-vivo fermentation experiments confirmed the IBS microbiota produced less butyrate but strikingly more sulphide. H₂S is now recognized to be an important neurotransmitter causing hypersensitivity to colonic distension and might be a mediator of the microbiota influence. Bile acids can also sensitize the gut to distension. Faecal primary bile acids are increased in both colonic IBD [24*] and IBS [25*] compared to healthy controls, possibly due to a reduction in metabolism caused by acceleration of colonic transit or by an abnormal microbiota. Interestingly, secondary bile acids exert an anti-inflammatory effect via the bile acid-specific membrane receptor TGR5, and the authors suggest that this loss of anti-inflammatory effects might be important for inflammation in IBD.

Microbiota may exert an influence on motility via activation of TLRs as shown by the finding that mice deficient in TLR4, a pathogen recognition receptor targeted to lipopolysaccharide (LPS), and its downstream signalling molecule MyD88 both show loss of nitricergic neurones and slower intestinal transit [26*].

Understanding of the role of microbiota in IBS has been summarized recently [7**], but the number of studies is limited. The largest study to date from Finland [27] found that by using 129 genus-like markers within the 16sRNA gene, they could distinguish the microbiota of healthy individuals from IBS patients. Firmicutes dominated, but the ratio of Firmicutes : Bacteroidetes was higher in IBS who had lower levels of Bifidobacteria and a slight increase in Gammaproteobacteria. There was a negative correlation with overall symptom score for some genus-like groups such as *Faecalibacterium* and *Einbacterium rectale*, but positive correlation with Gammaproteobacteria. Jeffery et al. [28**] used a smaller Swedish cohort (37 mixed-type IBS patients compared to 20 healthy individuals). They identified three clusters of IBS patients based on their microbiota, two of which had significant increases in Firmicutes/Bacteroidetes ratio and were distinct from controls, whereas the other 15/37 clustered with the microbiota of healthy individuals. The rates of clinically
significant depression were 2/22 (9%) in the first two IBS groups, but 6/15 (40%) in those with normal-like microbiota, suggesting that patients without disordered microbiota may have more social and psychological drivers to their condition [28**]. A recent paediatric study found no differences in phyla between 22 children with IBS and diarrhoea, and 22 healthy controls [29], but with these numbers no definitive conclusions are possible.

**MANIPULATING THE MICROBIOTA: ANTIBIOTIC, PROBIOTIC, PREBIOTIC**

Two large randomized placebo-controlled trials in IBS patients showed a small therapeutic benefit with rifaximin, a nonabsorbable rifamycin antibiotic [30] (number needed to treat (NNT) = 10), suggesting that a disordered microbiota, or ‘dysbiosis’, might be important, although whether that dysbiosis is present in the small bowel or colon is unclear. A recent study examined the effect of two courses of ciprofloxacin in three individuals. Whereas overall numbers of bacteria fell 1–2 logs, some bacteria were suppressed, whereas others increased. The response was mostly short-lived, but some permanent changes were noted which caution against the widespread use of antibiotics [31]. Antibiotic treatment leads to an increase in antibiotic resistance phages [32*,33] and increased susceptibility to pathogens such as *Clostridium difficile*.

These complications have encouraged interest in alternative methods of manipulation, one of the most extreme being faecal transplantation. A recent systematic review reported 317 cases of recurrent *Clostridium difficile* infection treated across 27 case series with a success rate of 92% [33]. This has been confirmed by a recent randomized controlled trial (RCT) of nasojejunal faecal infusion against standard antimicrobial therapy [34**], which backed up impressive clinical outcomes (NNT <2) with data showing the recovery of patients’ microbiotal diversity to levels comparable with faeces donors. Transplantation of a normal microbiome to treat *Clostridium difficile* infection in an otherwise normal bowel aims to re-establish normal colonization resistance with a persistently altered microbiota. IBD and IBS are both, however, chronic diseases with persistent abnormalities of function which may well alter the transplanted microbiota making its establishment more difficult. A systematic review of the evidence base for faecal transplant in IBD found nine reports and no controlled trials [35], but showed some promising data, though much less convincing than for *Clostridium difficile*, given the expected placebo response of 13–24% [36].

Devkota *et al.* [37**] elegantly showed that diets containing saturated milk fat, but not polyunsaturated vegetable oil, increased the percentage of total bile acids that were taurine-conjugated. This dietary change promoted the growth of the pathobiont *Bilophila wadsworthia*, which induced a colitis in mice deficient in IL-10 [37***]. The interaction between diet and microbiome is illustrated in humans by Smith *et al.* [38*] who found that Malawian children who developed kwashiorkor on their usual diet had a disordered microbiota compared to their healthy twins, and that a diet with higher energy availability could change the microbiota, preventing as well as resolving disease. Wu *et al.* [39] showed that dietary change can alter the microbiome within a day, but that enterotype is more stable and correlates better with long-term dietary habits.

Children in rural Africa have a microbiota very significantly different to that of urban European children [40] related to the substantially greater fibre intake (predominantly the nonabsorbable xylans and cellulose) and reduced dairy and meat intake in Africa. The discovery that SCFAs could regulate the enteroendocrine and immune response via G-protein-coupled receptor 43 (GPR43/FFAR2), and that oral acetate could attenuate DSS colitis which was worse in the absence of communal microbiota [41], indicated that these microbial metabolic products exert an anti-inflammatory effect. This is mediated via the GPR43 receptor as the attenuation was absent in *Gpr43* knockout mice. SCFA are also known to affect enteric neurons, increasing cholinergic neurones and increasing motility [42]. An RCT of 30 days of a poorly digested carbohydrate supplement in obese women showed a significant increase in stool Firmicutes, including *F. prausnitzii*, with a reduction in Propionibacteria and some *Bacteroides* [43*]; however, there was no effect on the numerous metabolic parameters measured. There has been a report of the benefit of an elemental diet in nine patients with pouchitis with nonsignificant increase in selected Firmicutes, but this needs repeating with larger numbers [44]. One case report showed normalization of microbiota in a patient with Crohn’s disease treated successfully with an elemental diet of protein and fat, encouraging further larger studies [45].

Patients with IBS often report improvement with diets excluding high-fibre foods, and diets reducing intake of fermentable carbohydrates, labelled low FODMAP diets, have proved popular [46]. There may be a role for restricting FODMAPs after gastroenteritis since mice were less able to clear a pathogen and reconstitute their microbiota when polysaccharides were available than when they were replaced with simple sugars, a manoeuvre which may force commensals to compete with pathogens [47**]. Kashyap *et al.* [48**], using gnotobiotic mice with and without a humanized microbiome,
showed not only that microbiota alter transit but that transit alters the microbiota, making it clear that inferring causation from association will be particularly problematic in this field.

**CONCLUSION**

The microbiome is potentially readily manipulated for therapeutic benefit. However, translating preclinical progress to bedside intervention will need larger studies than are currently available to provide the needed power, given the large number of variables which alter outcome. Since diet seems to be the overriding factor in the long-term stability of the microbiota, methodologically rigorous evaluation of diet will be important. Both IBD and IBS have uncertain cause and the insights gained from this investigative process will undoubtedly shed new light on their genesis and reveal new approaches to prevention, as well as treatment (Fig. 1).

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**Conflicts of interest**

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There are no conflicts of interest.

**REFERENCES AND RECOMMENDED READING**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


Gastrointestinal hormones


How fructans influence the microbiome.
Describes how the microbiome recovers from and expels a pathogen, and the role of dietary carbohydrate.
Explores the interactions between gut transit, microbiota and dietary carbohydrates.