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Inflammatory bowel disease and the gut microbiota

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Inflammatory bowel disease (IBD) is a group of immune-mediated disorders characterised by a chronic, relapsing-remitting inflammation predominantly affecting the gastrointestinal tract. IBD is incurable, affecting people in their most productive years. IBD is historically seen as a disease of Westernised nations although in recent times other countries have seen an exponential rise in cases. Although the exact pathogenesis remains unclear, evidence suggests that microbiota changes play a critical role in IBD pathogenesis. Over the past two decades, IBD has become one of the most studied human conditions linked to the gut microbiota. However, deciphering the intricate link between the gut microbiota and therapeutic efficacy remains elusive. This review will summarise the current evidence relating to the gut microbiota and its involvement in IBD pathogenesis as well as the impact of IBD treatments including pharmaceutical-, nutraceutical- and microbial-focused regimens on the gut microbiota.

Key words: Inflammatory bowel disease: Gut microbiota: Therapeutics: Microbial manipulation

Inflammatory bowel disease (IBD) is comprised of several similar yet clinically distinct entities. The vast majority can be characterised as ulcerative colitis (UC) or Crohn's disease (CD)[1]. A small number, approximately 10 % of cases have features of both and are classified as 'undifferentiated IBD', which is more common amongst children[2]. The intestinal inflammation which occurs in IBD results in typical symptoms of abdominal pain, diarrhoea and passage of blood or mucus per-rectally. Sufferers generally will experience attacks or 'flares' of disease activity which are interspersed between the periods of relatively symptom-free remission. If untreated, these can culminate in the loss of intestinal function, resulting in complications such as malnutrition.

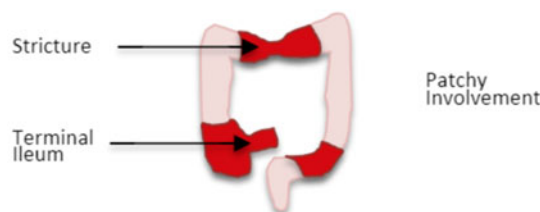CD is characterised by inflammation that can involve the entire thickness of the gastrointestinal tract, so-called transmural inflammation (Table 1) and may involve the entire gastrointestinal tract from mouth to the perianal area. Inflammation is discontinuous, resulting in 'skip lesions' where active disease is interspersed within the patches of normal-appearing bowel. The most commonly affected area is the ileum and proximal colon. The transmural inflammation can give rise to fistulous tracts that traverse through the bowel wall, sometimes into adjacent bowel or organs. This can manifest in intestinal perforation which requires surgical intervention. The intestinal lumen can also become narrowed due to inflammation or the formation of fibrotic strictures, resulting in bowel obstruction. Acute complications of CD require surgery in 6–16 % of cases, with up to 40 % of patients requiring further resections within 10 years due to disease recurrence[3]. Repeated resection can lead to short gut syndrome

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Table 1. Comparison of UC and CD location, inflammation, presentation and treatment

	UC	CD
Location	Always affects rectum extending proximally to involve the large bowel in continuous fashion Does not affect small bowel or upper gastrointestinal tract	Anywhere in gastrointestinal tract Frequently affects terminal ileum and perianal skin Patchy, discontinuous inflammation aka 'Skip lesions'
Depth of inflammation	Confined to colonic mucosa	Transmural
Complications	Toxic megacolon Colorectal cancer	Intestinal perforation, abscess, strictures and fistulae (collection between bowel and adjacent structures) Colorectal cancer (less risk than UC)
Extraintestinal manifestations	Skin: erythema nodosum, pyoderma gangrenosum Eye: uveitis, scleritis, episcleritis Arthritis Sclerosing cholangitis Thromboembolism	
Medical treatment	*Mild disease:* 5-aminosalicylates, steroids *Moderate to severe disease:* Steroids Immunomodulators Biologics	Corticosteroids Immunomodulators Biologics

with profuse chronic diarrhoea and nutrient deficiencies[4]. One of the most debilitating aspects of CD is involvement of the perianal area by abscesses and fistulae, which are painful and often disfiguring.

In contrast, the inflammation which occurs in UC is limited to the mucosal layer of the colon (Table 1). UC invariably involves the rectum and extends in a proximal and continuous fashion to other regions of the colon. A potentially catastrophic variant of UC is acute severe disease which is defined by a set of clinical and laboratory parameters known as 'Truelove and Witts Criteria'[5]. Acute severe ulcerative colitis affects 15–25 % of UC patients during their lifetime and carries a 50 % likelihood of requiring total colectomy within 3 years of acute severe ulcerative colitis diagnosis[6]. Chronic colonic inflammation can result in dysplasia which can progress to malignancy. This occurs to a greater degree in UC compared to CD. The cumulative risk of colorectal cancer in UC patients is 7·6 % after 30 years of disease[7], although increasingly there is a recognition that risk is associated with chronically active disease as much as duration of disease[8].

Both UC and CD are associated with pathology in other organ systems, these are known as extra-intestinal manifestations[9]. Musculoskeletal extra-intestinal manifestations include sacroiliitis, ankylosing spondylitis, peripheral arthropathy and osteoporosis. In the eye, episcleritis, scleritis and uveitis have been described. Cutaneous manifestations include erythema nodosum and pyoderma gangrenosum. Primary sclerosing cholangitis is the most common liver disease specific to IBD, affecting 4–5 % of patients, and is more commonly associated with UC[10].

Role of the microbiome in inflammatory bowel disease

As with most immune-mediated diseases, IBD is considered an idiopathic condition, with no clear aetiologic agent[11]. After decades of epidemiologic, genetic, laboratory and clinical studies, the complex interactions between factors influencing IBD pathogenesis are only beginning to be understood. Abundant evidence now suggests that a dysbiotic intestinal microbiota, characterised by an altered ratio of pro- to anti-inflammatory microbes plays a central role in initiating and perpetuating intestinal damage[12]. Genetic influences including host genetic polymorphisms in a number of genes that are involved in microbial recognition and processing, have also been identified[13,14]. Environmental factors including lifestyle, diet and medications further affect the balance, often through their impact on intestinal microbiota composition[12,15,16]. It is now generally accepted that IBD results from a 'perfect storm' of interactions between a dysbiotic microbiota, aberrant immune system and environmental exposures within a susceptible host (Fig. 1)[17].

The human gut hosts approximately 10^{14} bacteria comprising up to 1000 different species, along with viruses, fungi and other microorganisms[18]. The
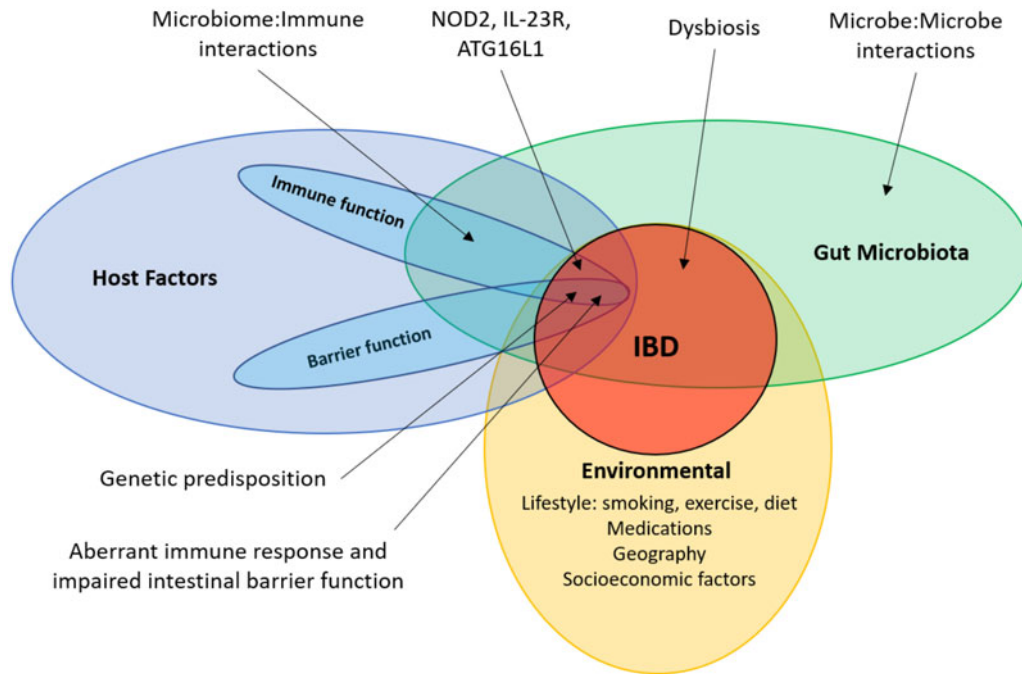
Fig. 1. Interactions between the gut microbiota, host and environmental factors in the pathogenesis of inflammatory bowel disease (IBD). The gut microbiota, host and environmental factors modulate and affect each other to form a physiological balance. In IBD, this balance is disturbed e.g. dysbiosis of the gut microbiota which leads to an aberrant immune response. Adapted from Hold *et al.*[94]. ATG16L1, autophagy related protein 16-like 1; IL-23R, interleukin-23 receptor; NOD2, nucleotide-binding oligomerisation domain-containing protein 2.

collective genome of these microbes is referred to as the microbiome, which contains 100-fold more genes than the entire human genome[19]. The intestinal microbiota is in continual contact with the host immune system, generating inflammatory responses to eliminate pathogens as well as promoting systemic tolerance to the collective microbiota[11,20,21].

Evidence to demonstrate the role of the microbiota in IBD pathogenesis is multifaceted. Animal studies have played a significant role in driving forward our understanding of IBD pathogenesis, especially murine colitis models. Several elegant studies have demonstrated the absolute requirement of the gut microbiota in the development of spontaneous colitis in genetically susceptible animals[22–24]. In the clinical arena, the effectiveness of antibiotics, such as rifaximin, in reducing intestinal inflammation have also been repeatedly demonstrated for some aspects of IBD management including in reducing recurrence of CD after surgical resection[25]. They are also the mainstay of treatment for inflammation of the ileal pouch after an IBD patient undergoes proctocolectomy[26]. In patients with CD, diversion of the faecal stream proximally to the inflamed mucosa has also been shown to result in the reduction of inflammation and induction of healing in the excluded parts of the gut, whereas relapse occurs with the restoration of faecal stream and re-exposure to luminal contents[27,28]. However, whether dysbiosis is the cause, result, contributor or 'innocent bystander' of aberrant inflammation remains unclear and remains the topic of ongoing intense study.

The cyclical relapsing and remitting nature of IBD also fuelled the hope that a microbial culprit was waiting to be found; akin to the *Helicobacter pylori*:peptic ulcer story. Many organisms were proposed but those deemed to have been of most interest over the years include *Mycobacterium paratuberculosis*, *Escherichia coli* and various *Helicobacter* and *Campylobacter* species[29–32]. Studies however failed to definitively attribute cause/ association of a single culprit as the cause of disease with recognition that the complexity of the lower intestinal microbiota meant it was equivalent to 'looking for a needle in a haystack'. An important shift in recent times, facilitated by the advancement of next-generation sequencing techniques and culturomics, has been the move towards identification of all species within an ecosystem's microbiota. As a result, instead of focusing on a single species, the current research paradigm considers the entire microbial ecosystem as the potential culprit. This paradigm shift has allowed a picture of the IBD microbiota to emerge; which is typified by *lower microbial diversity*, *altered microbial composition* and *microbial community instability* compared to the non-IBD subjects.

Halfvarson *et al.* performed one of the pioneering longitudinal cohort studies evaluating intestinal microbiome composition of individuals with CD, UC and healthy controls[1]. Stool samples were collected every 3 months for up to 2 years and microbiome composition was determined by 16S rRNA analysis. In keeping with previous studies, microbial composition in controls and IBD formed distinct clusters, with ileal CD patients having
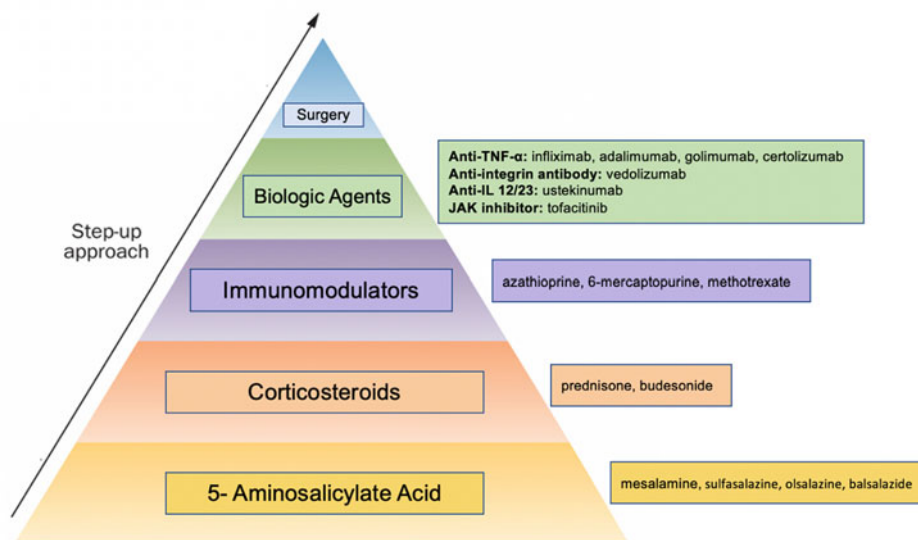
Fig. 2. 'Step up' approach to inflammatory bowel disease (IBD) therapeutics where there is escalation from safer and low-efficacy drugs, such as 5-aminosalicylic acid to more potent drugs such as corticosteroids, immunomodulators and biologic agents based on IBD severity. Adapted from Aloi et al.[47]. JAK, Janus kinase.

the most divergent microbiota profiles compared to healthy controls. Unique insights emerged when patients were evaluated over time. First, although microbiome profiles from healthy controls varied over time, they did so within a relatively limit range. In contrast, IBD microbiomes fluctuated to a much greater extent, occasionally entering the 'healthy' zone but not generally remaining there. This observation emphasises that IBD is characterised by a volatility that is not found in the healthy gut. During the study period, seven IBD patients experienced a flare of their disease symptoms and received a course of oral corticosteroids. These patients had greater fluctuations than those on stable medication. However, as no additional samples were collected at the time of the flare, it remains unclear whether there was a specific microbial signature associated with increased disease activity.

Other longitudinal studies have explored how microbiota networks relate to long-term disease severity and responsiveness to different IBD treatments[12,21,33–36]. As the number of these studies increases and the repertoire of multi-omics analysis tools expands, the complexity of the role of the microbiome in IBD is increasingly unravelling. Although changes in microbiota composition are seen based on geography, age and diet, the overarching microbiota changes in studies indicates a global reduction in microbial richness in IBD cohorts with clear separation of CD from healthy controls whilst UC is more heterogenous[37–39]. In addition, the metabolites, produced by microbes, are increasingly being shown to play a pivotal role in gut homoeostasis through alteration of signalling pathways, immune functioning as well as antimicrobial activity[40,41]. In both CD and UC, a greater level of dysbiosis is seen in patients experiencing a disease flare compared to patients in remission but consistent differences are not seen between inflamed/uninflamed tissue from the same patient indicating that microbial changes are a field change rather than simply due to the presence of inflammation.

Take home messages that have come out of multi-omics assessment of IBD patient cohorts include the need to explore wider patient sample sets including serum, stool, biopsies and urine. There is also a growing appreciation that analytical approaches may well need to differ between UC and CD. This is due to the current limited identification of UC-specific biomarkers specific taxa, metabolomic molecules and diagnostic biomarkers, compared to CD, than has been identified by current approaches. The other exciting prospect to come out of multi-omics analysis datasets is the ability to interrogate microbiota changes in response to treatment regimens. Multiple studies have demonstrated the impact of microbes on drug availability and treatment efficacy in many immune-mediated disorders[42–45]. Given the central role of the gut microbiota in IBD pathogenesis, understanding the impacts of therapeutics on gut microbiota and conversely, how the microbiota is related to treatment outcomes is essential to evaluate the role of the gut microbiota as a predictive biomarker for treatment response.

Inflammatory bowel disease treatment options

IBD treatment aims to achieve disease remission and mucosal healing in addition to a reduction in symptoms[46]. A 'step-up' approach to treatment is often used which is based on escalation of drugs from those with a better safety profile and cost-effectiveness but lower efficacy such as 5-aminosalicylic acid (5-ASA) to those that are more potent but with greater risk of adverse effects such as corticosteroids, immunomodulators and biologic agents (Fig. 2)[47]. However,

increasingly there is a recognition that risk is associated with chronically active disease as much as duration of disease[8,47]. Risk profiling of disease patterns is also used to accelerate step up therapy in patients who have high risk disease.

Therapeutic approaches to ulcerative colitis management

First-line UC treatments to induce remission are 5-ASA and/or corticosteroids. Patients who do not respond are considered at high-risk for colectomy and are 'stepped up' to more aggressive therapies involving immunomodulators or biologic agents such as anti- TNF-α agents, anti-integrin antibodies or Janus kinase inhibitor (Fig. 2). Anti-TNF-α agents are also used as both induction and maintenance therapy. These agents have been shown to alleviate symptoms, induce mucosal healing, reduce hospitalisations and colectomies. However, loss of response often as a consequence of immunogenicity affects about 30 % of patients within the first year of therapy[48]. Therefore, combination therapy with an immunomodulator is preferred given its ability to suppress antibody formation. Vedolizumab is the second-line agent for induction of remission in patients non-responsive to anti-TNF-α agents. It has good efficacy, safety profile and lower rates of immunogenicity[49]. Thus, it can often be used without immunomodulator agents.

Therapeutic approaches to Crohn's disease management

It is postulated that there is a short window of opportunity for the treatment of CD that can prevent irreversible bowel damage, hospitalisations, surgeries and disabilities[50]. On this basis, CD patients with clinical indicators for poor outcomes: younger age of onset, perianal disease or extensive anatomic involvement are treated most aggressively. Current evidence suggests that response/remission rates are higher if a biologic agent is given within 2 years of disease onset[51] and early use of biologics is associated with significantly reduced rates of hospitalisations, surgeries and complications[52]. Patients who have treatment failure with anti-TNF-α agents can be switched to ustekinumab or vedolizumab.

Changes in gut microbiota with inflammatory bowel disease therapeutics

5-ASA decreases inflammation through inhibition of NF-κB and pro-inflammatory eicosanoid production, and activation of PPAR-γ[53]. However, its effects on microbiota composition remain unclear. A prospective cohort study by Morgan *et al.*[34] observed significant reductions in *Escherichia/Shigella* abundance and modest increases in *Enterococcus* abundance in stool samples of one hundred thirty-one IBD patients on mesalamine[34]. These findings were corroborated by Xu *et al.*[54], who studied microbiota composition between the treatment-naïve UC cohort and 5-ASA-treated patients. They observed that there was a lower abundance of *Escherichia/Shigella* and an increased abundance of Firmicutes in patients on 5-ASA medication[53]. Subsequent administration of 5-ASA for at least 6 months to treatment-naïve patients

validated these specific alterations. However, both studies were limited to a single time point. Given that the IBD gut microbiota is typically more dynamic than healthy controls, it is essential to extend assessments of the microbiota over the course of treatments to identify whether these changes are sustained and treatment specific, or whether they simply reflect alterations in the degree of intestinal inflammation.

More recently, Schirmer *et al.*[55] investigated changes in the gut microbiota of four hundred and five paediatric UC patients, treated with 5-ASA and corticosteroids, by comparing treatment-naïve baseline to week 4 samples grouped by treatment type and remission status. Eleven operational taxonomic units (OTUs) were associated with 5-ASA use whereas forty-seven OTUs were linked to corticosteroid treatment. Differences in bacterial abundance associated with remission following 5-ASA medication included OTUs belonging to *Oscillospira*, *Eikenella*, *Rothia mucilaginosa*, *Clostridiales* and *Fusobacterium*. OTUs associated with corticosteroid response included Actinomyces which increased in the week 4 remission group but decreased in those with sustained disease[55]. The inverse was observed for a *Clostridium* OTU. Additionally, species including *Bifidobacterium*, *Fusobacterium*, *Dialister*, *Blautia producta* and *Eikenella*, showed significant differences in their mean abundances between the remission and no-remission groups[55]. These findings differ from the previous cross-sectional analysis by Morgan *et al.*[34], who observed that *Enterococcus* was the only genus altered during corticosteroid treatment, therefore suggesting that treatment response can be dependent on other factors including baseline microbiota composition. Nevertheless, this study was limited since the changes in microbial composition and remission status beyond 4 weeks are unknown. Furthermore, findings in the paediatric cohort may not be generalisable to the adult IBD population. Therefore, additional longitudinal prospective studies are needed.

Biologic agents

TNF-α is a pro-inflammatory cytokine produced by macrophages during acute inflammation. TNF-α signals through two transmembrane receptors, TNFR1 and TNFR2, and regulates a number of critical cell functions including cell proliferation, survival, differentiation and apoptosis[56]. TNF-α is considered a 'master-regulator' of inflammatory cytokine production because of its pivotal role in orchestrating the cytokine cascade in many chronic inflammatory conditions such as rheumatoid arthritis, psoriasis and IBD. Since the 1990s, the development and use of drugs which inhibit TNF-α action has revolutionised the management of these immune-mediated diseases[57]. Infliximab, adalimumab, golimumab and certolizumab are TNF-α inhibitors used in the treatment of IBD.

Treatment with TNF-α inhibitors has been shown to have a significant impact on faecal microbiota community profiles in CD. In both paediatric and adult studies, a compositional shift towards a healthier gut microbiota has been seen within 6 weeks of treatment. In addition, increases in SCFA producing bacterial species including

Roseburia, *Odoribacter*, *Fusobacterium* and *Prevotella* have been reported with the more pro-inflammatory *Klebsiella*, *Escherichia* and *Enterococcus* genera decreasing significantly in patients achieving remission[39,58–60]. In general, TNF-α inhibitors treatment success is associated with higher bacterial diversity/richness at baseline and a decrease in Actinomyces and increase in *Lactococcus* and *Roseburia*, following 6 weeks of drug. The findings have been broadly confirmed in both adult and paediatric studies. Kolho and Sipponen[61] assessed the effect of various TNF-α inhibitors in paediatric IBD patients, demonstrating that by week 6 of TNF-α inhibitor treatment, microbial diversity amongst patients and their similarity to the microbiota of controls increased in the responder group, but not amongst non-responders[62]. Furthermore, the increase in microbial diversity also correlated with an improved long-term outcome. Interestingly, there were six groups of bacteria whose abundance at baseline predicted the response to infliximab. These included genus-level groups *Bifidobacterium*, *Clostridium colinum*, *Eubacterium rectale*, uncultured *Clostridiales* and *Vibrio*. These bacteria have been associated with health as they are known to have immune-stimulatory properties and are found in high abundance during early childhood[63]. Responders also had a lower abundance at baseline of *Streptococcus mitis*. High microbial diversity at levels equivalent to controls was significantly associated with a sustained therapeutic response and lower calprotectin levels at 3 months, thus suggesting the potential of microbiome analysis to predict treatment outcomes. The recent longitudinal Swiss IBD cohort study also found that amongst CD patients receiving TNF-α inhibitors, increased *Bifidobacterium*, *Collinsella*, *Lachnospira*, *Lachnospiraceae*, *Roseburia*, *Eggerthella* taxa and reduced *Phascolarctobacterium* were associated with treatment success[12]. One study also observed microbiota changes associated with infliximab discontinuation[64]. In a study of thirty-three CD patients in stable remission on combined immunomodulator and infliximab therapy, infliximab was discontinued as a part of planned therapy de-escalation with faecal microbiota composition evaluated at baseline, 8 weeks, 6 months and at 18 months, by which time nineteen patients (58 %) had relapsed. There was no significant fluctuation in faecal microbiota composition across time-points in either relapsers or non-relapsers; there was also no correlation between microbial signals and inflammatory markers. Bacterial signals which corresponded with relapse however included reduced the numbers of *Faecalibacterium prausnitzii*, Bacteroides members and *Clostridium coccoides*[64].

In UC, Magnusson et al.[60] studied the microbiota composition of fifty-six biologic-naïve adult UC patients who commenced anti-TNF therapy. Based on stratification into responders *v.* non-responders after 12–14 weeks of treatment they found that responders had lower dysbiosis indices and a higher abundance of *F. prausnitzii* at baseline compared to non-responders[60]. Furthermore, a longitudinal increase in *F. prausnitzii* was observed in responders at weeks 2 and 6. Additionally, the study analysed microbiota and proteomic data from faecal and mucosal biopsy samples respectively and found that at baseline, responders had detectable expression of several antimicrobial peptides or proteins while non-responders had expression of a protein which inhibited antimicrobial peptide expression. This difference in antimicrobial response was postulated to be a dysbiosis indicator and higher baseline expression of these proteins was potentially a predictor of anti-TNF-α therapy response.

Other biologic agents: anti-integrin antibody, anti-IL-12/ IL-23 antibody

Vedolizumab is a humanised anti-α₄β₇ integrin monoclonal antibody that selectively blocks trafficking of memory T cells to inflamed gut tissue by inhibiting the α₄β₇-mucosal addressin cell adhesion molecule-1 interaction[65]. Approved for treating patients with moderately to severely active UC and CD, vedolizumab is generally considered safer than other biologics due to its gut-specific mode of action.

Recently, Ananthakrishnan et al.[42] evaluated the effects of vedolizumab on gut microbiota composition in forty-three patients with UC and forty-two patients with CD. In CD patients, five taxa significantly decreased in relative abundance between baseline and week 14 in patients achieving remission. These taxa include *Bifidobacterium longum*, *Eggerthella*, *Ruminococcus gnavus*, *Roseburia inulinivorans* and *Veillonella parvula*. In UC, only one taxon, *Streptococcus salivarius*, significantly increased in abundance in patients who did not achieve remission. The α diversity was significantly higher whereas the β diversity was lower in CD patients at baseline achieving remission by week 14. In particular, *R. inulinivorans* and a Burkholderiales species were significantly more abundant at baseline in patients achieving remission. Additionally, responders at week 14 demonstrated greater persistence of their microbiota changes at 1 year compared to non-responders, suggesting that early changes in microbiome could be an indicator of sensitivity to treatment and initial response. When the authors examined the trajectory of metabolic pathways during vedolizumab therapy, they noted more pronounced trends compared to microbial composition. This suggests that understanding the net metabolic and immunologic effect of resident microbiota, rather than that of single organisms is key to unravelling the mechanism of intestinal damage in IBD.

Another newer biologic agent to enter the scene of IBD treatment is ustekinumab, an inhibitor of IL-12 and IL-23. These are related cytokines that have been implicated in the pathogenesis of several immune-mediated disorders including IBD. They are heterodimers made up of a common p40 subunit complexed to unique p35 (IL-12) or p19 (IL-23) sub-units. Ustekinumab is a human monoclonal antibody that specifically binds the p40 subunit of IL-12/23, thus preventing IL-12 and IL-23 from binding to their cell surface receptor complexes, thereby blocking the T helper (Th) 1 (IL-12) and Th17 (IL-23) inflammatory pathways[66]. Ustekinumab has proven efficacy in the treatment of moderate to severe CD and UC which are refractory to other therapies. Its long-term safety of doses used

in the treatment of IBD is not yet well-established, but is believed to be similar to TNF-α inhibitors[67].

Doherty *et al.*[68] conducted a multicentre randomised placebo-controlled phase 2B trial which analysed microbiota changes associated with ustekinumab treatment. The findings followed the trend reported in the majority of anti-TNF-α studies, with an increase in microbial diversity particularly SCFA-producing bacteria in treatment responders. Following 6-weeks of treatment, the relative abundance of *Ruminococcaceae*, *Faecalibacterium*, *Blautia*, *Clostridium* XIVa and *Roseburia* was higher, and the proportion of *Shigella* and *Escherichia* was lower amongst ustekinumab responders compared to non-responders. The α-diversity measures were also increased amongst ustekinumab responders at week 22, whereas no significant change was measured amongst non-responders or those who received placebo. In addition, baseline microbiota profiles were shown to predict response to ustekinumab. Baseline α-diversity amongst ustekinumab-treated patients in remission at 6 weeks was 1·7 times higher compared to those with persistent CD activity. *Faecalibacterium* and *Bacteroides* were significantly more abundant at baseline amongst patients who achieved in remission 6 weeks compared to those who did not.

Total parenteral nutrition and exclusive enteral nutrition

Dietary antigens have potential to stimulate mucosal immunity, therefore eliminating them through bowel rest with has been utilised as an early therapy in IBD[69]. Total parenteral nutrition where the patient is fasted whilst a mixture of lipids, glucose, amino acids, salts with added dietary minerals and vitamins is administered intravenously, emerged in the 1980s as an important strategy for the treatment of moderate to severe CD. In a prospective study, CD patients were treated with total parenteral nutrition and bowel rest. The majority achieved initial remission, but relapse was common once food was re-introduced[70]. Conversely, there are very few data regarding the effectiveness of enteral therapy for UC. A small randomised trial of hospitalised patients with severe UC did not find any differences in response rates to corticosteroid therapy with a polymeric diet compared with total parenteral nutrition[71].

In recent decades, exclusive enteral nutrition (EEN) has superseded total parenteral nutrition as a safer dietary strategy for treating CD. EEN utilises diets composed of elemental, semi-elemental or defined formulae. It has proven efficacy for inducing remission and is often utilised as first-line therapy, particularly for paediatric patients with CD[72,73]. EEN's utility in UC is less well-established.

Despite its efficacy for CD, the mechanism of EEN action has not been fully characterised. Interestingly, there does not appear to be major differences in EEN efficacy based on the composition of the formula, with a Cochrane meta-analysis finding similar efficacy of formulas with variable degrees of protein hydrolysis in treating CD[74]. Several hypotheses have been proposed to explain the efficacy of EEN for CD. The most likely mechanisms include direct anti-inflammatory effects and improvement of intestinal barrier function. Modulation of gut microbiota has also been proposed although current data are sparse. In a small case series of nine paediatric CD patients treated with polymeric EEN, all experienced significant shifts in intestinal microbiota composition[75]. Another study measured the faecal abundance of five key bacterial taxa from CD patients treated with EEN compared with healthy controls on a standard diet. At baseline, bacterial diversity present was comparable. At week 8 of follow-up, CD patients displayed reduced microbial diversity, and this persisted for several months after cessation of EEN[76]. The authors observed a paradoxical reduction in 'protective' gut bacterial species such as *F. prausnitzii* and metabolites including butyrate usually associated with reduced gut inflammation. Similar reduction in *F. prausnitzii* abundance was reported amongst healthy volunteers placed on EEN[77]. These findings challenge the previous notion that increased microbiome diversity and higher numbers of *F. prausnitzii* are central to gut health.

To explore associations between the gut microbiota during EEN, Quince and colleagues utilised 16S rRNA sequencing and shotgun metagenomics to determine microbial composition of faecal samples from CD and healthy children. From the CD patients, faecal samples were collected before and during EEN, and after return to habitual diet. Microbial diversity was lower in CD than in controls before EEN. During EEN, the microbial diversity of CD children faecal samples further decreased. The reduction in diversity became apparent after only 15 d on EEN with lowest microbial diversity levels observed by 30 d of EEN treatment. A slight recovery towards the end of EEN and complete recovery of microbial diversity measures to pre-treatment levels were seen when patients returned to a regular diet. During EEN, the microbial community structure became less similar to that of controls compared to pre-EEN samples. The vast majority of changes represented a reduction in relative abundance, with some of the most negatively impacted genera being *Bifidobacterium*, *Ruminococcus* and *Faecalibacterium*. Their abundance was already lower at baseline in CD children compared to controls. The only genus that increased with EEN treatment was *Lactococcus*. In terms of microbial metabolic pathways, the abundance of genes involved in biotin and thiamine biosynthesis decreased during EEN, indicative of a reduction in bacteria that bear genes encoding for these vitamins, such as *Bifidobacteria* and *E. coli* spp., or changes in the synthesis of SCFA that require these vitamins. Conversely, pathways involved in spermidine/putrescine biosynthesis increased[78]. These pathways are known to play a major role in cell growth, and are considered a marker of cell renewal and epithelial healing[79]. Tjellstrom and colleges also reported an increase in SCFA production amongst CD patients commenced on EEN[80].

In the recently published CD-TREAT study, Svolos and colleagues evaluated the effects of an individualised food-based diet, with similar composition to EEN, on the gut microbiota, inflammation and clinical response[81]. Using a combination of animal studies and human studies

including five children with relapsing CD, they created a solid food EEN by the exclusion of certain dietary components (e.g. gluten, lactose and alcohol) and matching of others (macronutrients, vitamins, minerals and fibre) as closely as possible using ordinary food. When compared to conventional EEN, the CD-TREAT diet induced similar effects on faecal microbiome composition, metabolome and mean total sulphide. Sulphides is a bacterial product known to break down mucus barrier and been linked to intestinal inflammation[82]. Similar effects on bacterial load reduction and SCFA composition were also comparable between the two diets. Amongst the children with CD, 8 weeks on the CD-TREAT diet led to clinical improvement in 80 % and clinical remission in 60 %[81]. Although a pilot study which requires extensive validation and extended timeframe evaluation, the landmark study clearly demonstrates how through careful consideration of nutrition it is possible to achieve equivalent if not superior effects on IBD symptoms, thus providing additional less toxic therapeutic options for disease remission and management.

Probiotics

The success of probiotics in the management of IBD ranges from mixed results to considerable potential and is dependent on the strains used and disease subtype targeted. The most encouraging studies have been in the non-pathogenic strain of *E. coli* Nissle 1017, as well as VSL#3, which contain four strains of *Lactobacilli* (*L. casei*, *L. plantarum*, *L. acidophilus* and *L. delbrueckii* subsp. *bulgaricus*), three strains of *Bifidobacteria* (*B. longum*, *B. breve* and *B. infantis*) and one strain of *Streptococcus* (*S. salivarius* subsp. *thermophilus*). These probiotics have been shown to be effective against recurrence of pouchitis after surgery, as well as in the induction and maintenance of remission in UC[83].

Faecal microbiota transplantation

In simple terms, faecal microbiota transplantation (FMT) replaces the subject's dysbiotic gut microbiota with microbes from 'healthy' donors. As early as 1989, FMT was being used to treat IBD, sometimes with dramatic responses. A systematic review, published in 2012, containing twenty-six subjects found that nineteen patients experienced symptomatic improvement, thirteen ceased taking IBD medications within 6 weeks and fifteen had no active disease 3–36 months following FMT[84].

Bennet and Brinkman's initial report documented the complete clinical remission of a case of UC for at least 6 months following FMT through retention enema[85]. A paediatric case series subsequently found that seven out of nine patients with mild-to-moderate UC disease activity experienced clinical improvement, and three achieved clinical remission within 1 week after a 5-d course of daily FMT enema[86]. In contrast, no clinical improvement was observed in two smaller studies[87,88].

More recently, following the landmark study by van Nood *et al.*[89] describing the superiority of FMT for the treatment of recurrent *Clostridioides difficile*

infection, a number of studies have been published describing FMT treatment in IBD. Interestingly, FMT does not appear to be effective in CD, with all positive studies assessing UC cohorts. Moayyedi and colleagues randomised seventy patients with active UC to receive weekly FMT or placebo enemas[90]. By week 7, clinical and endoscopic remission was achieved in nine of thirty-eight (24 %) patients in the FMT arm *v.* two of thirty-seven (5 %) in the placebo arm, with no difference in adverse event rates reported between the groups. Interestingly, seven of the nine patients in remission after FMT received faecal material from a single donor, highlighting the possibility of the FMT 'super donor'[90]. After 6 weeks of treatment, there was a statistically significant change in microbiota composition, with an increased microbial diversity in the FMT group compared with the placebo group. Donor stool enrichment for the family Lachnospiraceae and the genera *Ruminococcus* was associated with successful treatment. A further study by Rossen and colleagues randomised fifty UC patients to receive either donor or autologous (patient own) FMT by nasoduodenal infusion but the study failed to detect a difference. The clinical response rate was 52 % in the control and 43·5 % in the treatment group ($P = 0.58$). At 12 weeks after treatment, the diversity index amongst responders in both groups increased, whereas no change in diversity was detected amongst non-responders. Microbial composition of responders in the treatment group shifted towards that of their donors by week 12, with regain of Clostridium clusters IV, XIVa, and XVIII, and reduction in Bacteroidetes. Responders in the control group also displayed a change in microbiome composition, but unlike FMT-treated responders, this shift was mostly associated with an increase in abundance of Bacilli, Proteobacteria, and Bacteroidetes[91].

Two subsequent studies utilised pooled stool from healthy donors. Paramsothy and colleagues randomised eighty-one patients to receive donor stool or placebo[92]. The index dose was administered via colonoscopy, and this was followed by an intensive regimen of daily enemas for 8 weeks. The primary outcome of steroid-free clinical remission was achieved in 27 % of donor-stool recipients *v.* 8 % of those assigned placebo ($P = 0.021$), with no difference in adverse effects. Stool microbial profiles of patients after donor FMT shifted from a dominance of *Bacteroides* spp. to *Prevotella* spp., bringing them closer to the donor's profile. Several microbial taxa were associated with remission including *Barnesiella* spp., *Parabacteroides* spp., *Clostridium* cluster IV and *Ruminococcus* spp. Conversely, *Fusobacterium* spp. and *Sutterella* spp. were associated consistently with the lack of FMT response[92].

Costello and colleagues were the first to utilise anaerobically processed pooled healthy donor stools for FMT in seventy-three patients with mild to moderately active UC[93]. The rationale for this approach was that anaerobic processing helped to achieve a microbial profile which more closely mimicked intra-luminal conditions. With one colonoscopic FMT dose followed by two subsequent enemas, patients receiving donor stool

demonstrated higher rates of clinical and endoscopic remission at 8 weeks compared to the placebo arm which received autologous stool. A total of 42% of remitters also maintained steroid-free remission to 12 months. As expected, diversity increased following donor FMT and this was maintained up to 8 weeks following treatment. An increased abundance of *Anaerofilum pentosovorans* and *Bacteroides coprophilus* was strongly associated with disease improvement following donor FMT. SCFA levels were not significantly different between treatment groups at weeks 4 or 8 and did not predict clinical outcome[93].

Conclusions

We have come a long way in our understanding of IBD pathogenesis and the involvement of the gut microbiota, but there is still a long way to go and defining optimal treatment strategies for patients remains a challenge. Lessons learnt along the way include the need to appreciate the bigger picture by allowing the study design to capture the differing aspects of this complex disease. This includes the need to focus on well-phenotyped patient cohorts, use of prospective longitudinal cohorts, appreciation of inflammatory and treatment confounders as well as considering differences due to geography, age and diet. Even with the consideration of these requirements, there remains the need to ensure robust standardised scientific methodology is applied consistently to ensure the quality of the findings.

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