Microb Health Dis 2022; 4 (3): e776

A YEAR OF MICROBIOTA AND IBD – HIGHLIGHTS AND ADVANCES

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Abstract – The role of gut microbiota in the pathogenesis and disease course of IBD continues to gain increasing scientific interest. This review highlights important advances in the field via collation and discussion of relevant publications between April 2021 and March 2022. A literature search was performed using the PubMed online database and the following search terms: inflammatory bowel disease(s) (IBD); ulcerative colitis; Crohn's disease; microbiome OR microbiota. Exclusion criteria included non-English language publications, narrative reviews, case reports and case series containing less than 5 patients. 1180 articles were initially identified. Duplicates were excluded and abstracts imported into an online systematic review platform (www.rayyan.ai). Abstracts were screened by one of four independent reviewers to ensure relevance. Abstracts of equivocal importance were flagged for second independent review. Human studies and novel, high-quality and methodologically robust animal models demonstrating important therapeutic or mechanistic insight were selected. 287/1180 (24%) were included with only highlights chosen for discussion. Selected papers were divided into one of seven subheadings: pathophysiology, genetics, therapy, FMT, diet and prebiotics, paediatrics and the mycobiome. Whilst we discuss a number of important advances, there remains a need for further, prospective, controlled and preferably human, research that better defines the bidirectional relationship between the gut microbiota and the pathogenesis, disease course and treatment responsiveness of IBD.

Keywords: Microbiota, Microbiome, Inflammatory bowel diseases (IBD), Crohn's Disease, Ulcerative Colitis, Genetics, Diet, Faecal microbiota transplantation (FMT), Mycobiome.

IBD PATHOPHYSIOLOGY

Whilst a correlation between intestinal microbiota and inflammatory bowel disease (IBD) has frequently been reported, the precise role of microbiota in the pathogenesis of IBD remains unclear. There were a number of scientific advances in the previous year.

The mucosal layer (i.e., mucus layer, epithelium and lamina propria) forms a physical barrier between the immune cells residing in the lamina propria and the microbiota that inhabit the intestinal lumen. Nevertheless, microbiota is in close contact with host immune cells by the production of extracellular vesicles, metabolites and secretory enzymes[1-3]. Extracellular vesicles are bilayer phosphide-based spheres produced by Gram-negative bacteria that can traverse the mucus layer and epithelium to interact with the mucosal immune cells[4]. Gul et al[1], investigated the inflammation-association Toll-Like Receptor (TLR) pathways elicited by the proteins contained within the bacterial extracellular vesicles (BEVs) of *Bacteroides thetaiotaomicron*. To this end, a single-cell transcriptomics dataset comprising data from healthy

controls and (non-)inflamed colon tissue from ulcerative colitis (UC) patients was used[1]. More specifically, the effect of BEVs on five immune cells (cycling monocytes, inflammatory monocytes, healthy mucosa-associated dendritic cells and dendric cells) associated with an inflamed mucosa was determined. From this data, it was apparent that the interactions between BEVs from B. *thetaiotaomicron* and mucosal immune cells are diverse. They include the promotion of cycling monocyte cell division in healthy tissue and offer protection against oxidative DNA damage by stimulation of DNA repair activity in UC patients. The most interesting results were obtained for TLR4, which was predicted to be a potential target for BEVs in cyclin monocytes, macrophages and inflammation-associated dendritic cells. In cycling and inflammation-related monocytes, TLR4 expression was found to be related to UC. Based on an *in vitro* BEV-monocyte co-culture experiment, the ability of BEVs to bind TLR4 extracellularly was confirmed. Interestingly, in addition to the extracellular BEV-TLR4 receptor interaction, evidence was found to suggest an intracellular interaction between BEVs and TLR4 pathway members.

To elucidate the correlation between the microbiome and the pro-inflammatory cytokines IL-17 and IL-23 in UC, Dai et al[5], investigated the difference in microbial composition between colon biopsies from UC and healthy control patients. Based on 16S rDNA sequencing, the bacterial species *Enterococcus*, *Lactobacillus* and *Escherichia-Shigella* were found to be significantly increased in UC versus control colon biopsies. *Bacteroides* appeared to be numerically but not significantly decreased in colonic biopsies of UC patients compared to controls. Immunohistochemical staining was also performed to investigate the role of IL-17 and IL-23 on the most abundant bacterial species in these samples. The expression of both IL-17 and IL-23 was positively correlated with the abundance of *Enterococcus*, *Lactobacillus*, *Bifidobacterium* and *Escherichia-Shigella*. This may suggest that an IL-23/IL-17 axis might mediate the link between microbiota and the chronic inflammation observed in UC.

Environmental factors also have the potential to influence the host through the microbiome. Serum concentrations of the endocrine disruptor bisphenol A were found to be positively correlated to the presence of bacterial DNA in the blood and with faecal calprotectin levels in patients with active Crohn's disease (CD)[6]. It was suggested that bacterial DNA in the blood was partially caused by dysbiosis elicited through exposure to bisphenol A. Furthermore, in serum samples of CD patients the IL-23/IL-17A axis was only upregulated in the subset of patients in which bacterial DNA was present in the blood and was not related to disease activity. Since bisphenol A has also been associated with gut dysbiosis in mice[7], this endocrine disrupting agent could be an environmental factor that influences CD pathogenesis *via* the effect on the gut microbiome[6].

Based on a cohort of 32 patients with active UC and 23 healthy controls, Yang et al[2] investigated the relationship between bile acid derivatives found in faeces and inflammation in UC patients. The abundance of several faecal primary bile acids was significantly increased in UC patients compared to healthy controls. In contrast, a number of secondary bile acids (i.e., metabolised primary bile acids), were significantly decreased in UC patients. Based on the differential abundance of microbiota determined with 16S rDNA sequencing, the overall microbial diversity was decreased in UC patients versus healthy controls. Associations could be made between the presence or absence of certain bile acids and faecal microbial composition. The interaction between bile acids and UC was further corroborated by the positive correlation of taurocholic acid (TCA), glycocholic acid (GCA) and glycochenodeoxycholic acid (GCDCA) with IL-1α and TCA with TNF-α, but also through the negative correlation of numerous secondary bile acids with serum levels of inflammatory cytokines IL-1α, IL-1β, TNF-α and IL-6. These cytokines were also upregulated in the serum of UC patients. Due to the close relationship between bile acid abundance and the microbiota, it was hypothesized that the microbiota-induced changes of the bile acid composition influence inflammatory responses. More specifically, immunohistochemical staining indicated an upregulation of Takeda G-protein-coupled receptor 5 (TGR5) and downregulation of vitamin D receptor (VDR) in colonic biopsies of UC patients versus healthy controls. This led the authors to suggest that the mechanism of action involved TGR5 and CDR since they have previously been linked to intestinal inflammation through the regulation of the NF-kβ pathway.

Li et al[8] investigated the bile acid isoallolithocholic acid, an isomer of the secondary metabolite lithocholic acid. This isomer was found to increase the transcription factor

NR4A1-dependent expression of the forkhead box P3 gene (FOXP3), stimulating differentiation of naïve T cells to Treg cells. The abundance of isoallolithocholic acid, was significantly reduced in IBD patients compared to non-IBD controls. Moreover, the production of isoallolithocholic acid could be traced back to a gene cluster present in several species from the *Bacteroidetes* phylum, suggesting a mechanism via which these species suppress inflammation.

The mucin barrier plays an important role in the protection of the epithelial barrier against pathogens. Through their extended glycosylation, mucins are largely protected from degradation. However, the regulated degradation of mucins is part of the symbiosis between host and microbiota. Both commensals and pathogens can have the ability to degrade mucins through the expression of several enzymes[9]. Recently, significant progress in the degradation mechanics of O-glycan-linked sulphate has been made. Luis et al[3] identified a single sulfatase ($BT1636^{3S\text{-}Gal}$) that largely contributes to the degradation of O-glycan-linked sulphate groups by B. *thetaiotaomicron* through the examination of sulfatase activity on porcine-derived sulphated colonic mucins. In addition, an *in vivo* experiment in gnotobiotic mice was performed in which B. *thetaiotaomicron* expressing different sulfatases were inoculated and colonization fitness was evaluated. It was apparent that B. *thetaiotaomicron* only lacking $BT1636^{3S\text{-}Gal}$ led to a significantly reduced colonization fitness when compared to wild-type strains. Such discoveries help to better understand mucin degradation in IBD and provide possible therapeutic avenues to influence mucin degradation.

GENETICS

A whole-genome metagenomic shotgun sequencing revealed no significant differences between IBD-twins and healthy co-twins in relative abundance of faecal microbial species and transcriptional pathways. Interestingly, many of the shared species and pathways have been either previously linked to IBD or have known pro-inflammatory properties such as an increase in propionate and L-arginine degradation. The authors conclude that the IBD-like microbiome signature of healthy co-twins might predispose to the future onset of IBD in these individuals, however, longitudinal follow-up studies are required to confirm this[10].

Associations between SNP genotypes and the gut microbiota were investigated in the Kiel IBD family cohort, consisting of 256 families with 455 IBD patients and 575 first- and second-degree relatives. The researchers observed that genetic similarity for SNP rs11741861 is inversely correlated with overall microbiome similarity among IBD-discordant relatives. Furthermore, a genome-wide quantitative trait locus (QTL) linkage analysis revealed an association between 12 chromosomal regions and the Shannon index of α diversity as well as the abundance of one of the following 7 microbial genera: *Barnesiella*, *Clostridium_XIVa, Pseudoflavonifractor, Parasutterella, Ruminococcus, Roseburia* and *Odoribacter*[11].

Zhuang et al[12] investigated causal relationships between gut microbiota, microbial metabolites and IBD. An increased abundance of OTU10032 unclassified Enterobacteriaceae and the Enterobacteriaceae-related metabolites taurine and betaine was linked to a higher risk of IBD. On the contrary, the Erysipelotrichaceae family, the Actinobacteria class and Unclassified Erysipelotrichaceae were associated with a lower risk of IBD[12].

A host genome-wide RNA-seq analysis revealed that the transcriptomic profile of the mucosal rectosigmoid colon samples of UC patients in histological and endoscopic (histo-endoscopic) remission is similar to the profile of healthy controls and that this group of patients has a lower risk of relapse. Furthermore, the group of UC patients with histo-endoscopic activity had increased expression levels of multiple host genes implicated in humoral immune response, antimicrobial defense, chemokine and Th17 signaling pathway compared to UC patients in histo-endoscopic remission[13].

Another study investigated the relationship between the microbiome and the transcriptome in terminal ileal CD. The authors found that multiple genes belonging to the chemokine ligand, matrix metalloproteinase and interleukin families showed significantly different expression levels between inflamed and non-inflamed mucosa of patients with terminal ileal CD. The gut microbiome analysis revealed the presence of Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, and Fusobacteria in terminal ileal CD. A linear discriminant analysis

revealed that Pseudomonadales and Micrococcaceae were less abundant, whereas Ruminococcaceae, *Haemophilus*, Leptotrichiaceae, *Leptotrichia*, Burkholderiales and Comamonadaceae were more abundant in inflamed samples *vs.* non-inflamed samples of patients with terminal ileal CD. Interesting interactions between immune genes, transcription factors and gut microbiome included *IRF4* with gut microbes *Rhodococcus* and *Butyrivibrio* in inflamed mucosa. The transcription factors *VDR* and *ELK3* are found to be in a central position when linking microbiota and differentially expressed genes and are significantly increased and decreased in inflamed mucosa, respectively[14].

While many genetic mutations have been described in IBD patients, their function in disease pathogenesis is less well defined. Cheng et al[15], investigated the function of FUT2 polymorphisms on the microbiome and IBD pathogenesis. FUT2 encodes the α-1,2-fucosyltransferase, which is involved in the expression of the fucosylated ABO blood group antigens that can be employed as anchorage sites for bacteria. 16S rRNA sequencing was performed on faeces from 81 IBD patients being either homozygous for the functional FUT2 gene, homozygous for the non-functional FUT2 gene or heterozygous for the non-functional FUT2 gene. Several differences in microbial composition between the different test groups were found. Most notably, *Alistipes* and *Phascolarctobacterium*, possible inducers of cytotoxic T-cells, and *Erysipelotrichaceae* UCG-003, a possible inducer of Th17 cells, were significantly more abundant in patients homozygous for the non-functional FUT2 polymorphism. The authors support the hypothesis that reduced expression of functional α-1,2-fucosyltransferase could result in less available adhesion sites and thus alter the composition of gut microbiota. This alteration in microbiota composition could be a contributing factor in the pathogenesis of IBD.

Besides, an interesting finding from another transcriptome-wide association study encompasses the detection of the tissue-specific candidate FUT2 for the genus Bifidobacterium in transverse colon, which was also found to be associated with CD[16].

IBD THERAPY

Research into IBD therapies and their relationship to gut microbiota continue to be dominated by murine models of nutritional and traditional medicine or anti-inflammatory supplements. Only articles including human participants or novel therapeutics with mechanistic insights in high-quality animal models were chosen for discussion.

Of conventional IBD therapies, anti-TNF agents remain the most studied in relation to the gut microbiota. Sanchis-Artero et al[17] reported changes in gut microbiota before and after 6 months of anti-TNF therapy in a prospective, multicentre longitudinal study of 27 patients with CD. Using 16S rRNA sequencing on faecal samples, non-responders to anti-TNF demonstrated increased Proteobacteria phylum, particularly *Clostridium* and less alpha diversity when compared to anti-TNF responders. Using receiver operator characteristic (ROC) curves, the *F. prausnitzii*/*E. coli* (FE) ratio was the most accurate biomarker of anti-TNF response (AUC 0.87, $p < 0.001$). The potential for this measure to provide a reliable and early indicator of anti-TNF response, rather than being driven by presence or absence of inflammation alone, requires further evaluation. Busquets et al[18] also investigated microbial predictors of anti-TNF response in 38 patients with IBD by comparing 8 distinct microbial markers in responders compared to non-responders. Whilst there were no significant differences in individual pre-treatment qPCR microbial markers between groups, an algorithm based on the relative abundance of eubacteria, *F. prausnitzii*, *Methanobrevibacter smithii* and *Ruminococcus spp*. had a high capacity for predicting anti-TNF treatment response at 12 months (sensitivity 93%, specificity 100%).

Repurposing ursodeoxycholic acid (UDCA) for use in IBD was investigated by Wang et al[19] in an RCT comparing mesalazine *vs.* mesalazine plus UDCA in adult patients with UC. The 20 patients receiving combination mesalazine + UDCA had superior clinical response, lower serum IL-23 and Il-17 levels and a lower proportion of Proteobacteria on 16S rRNA sequencing of faecal samples when compared to the 20 patients receiving mesalazine monotherapy. The authors correctly acknowledge that the improved clinical response may be independent of the favourable downregulation of the IL-23/IL-17 inflammatory axis and reduction in

Proteobacteria. Similarly, the anti-inflammatory effects of the established mood-stabilising agent lithium carbonate was investigated in a murine model of DSS-induced colitis. Lithium has been previously shown to modify regulatory T cell activity and inhibit GSK3β resulting in activation of the anti-inflammatory Wnt/β-catenin pathway[20-22]. In a series of experiments, Huang et al[23] demonstrated that, in addition to improving macroscopic and histological colitis, lithium carbonate treatment improved microbial alpha diversity and upregulated short-chain fatty acid (SCFA)-producing bacteria, especially *Akkermansia muciniphila*, with a subsequent increase in acetate and propionate production. Increased abundance of *A. muciniphila* was also observed in 18 lithium-treated patients without IBD compared to 18 matched non-lithium treated controls without IBD. To investigate the effect of this species, oral supplementation with *A. muciniphila* in a second DSS-induced colitis model also downregulated inflammatory cytokine expression and improved histological disease severity compared to controls.

Despite conflicting evidence, cannabinoids as anti-inflammatory therapies in IBD are gaining increasing interest. The two most common cannabinoid receptor ligands are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). In murine models, activation of cannabinoid receptors has been shown to protect against experimental colitis[24,25]. However, human trials have not shown convincing objective therapeutic effects[26,27]. In a series of experiments published in the Journal of Crohn's and Colitis, Becker et al[28] demonstrated that THC, but not CBD, ameliorated inflammation in both DSS- and TNBS-induced colitis. However, this was independent of the microbiome and metabolome. Whilst the THC group had higher levels of acetate, propionate and butyrate in their caecal contents when compared with controls, faecal transfer from THC treated mice to naïve mice pre and post induction of DSS colitis did not reduce colitis severity. Instead, the authors reported multiple mechanisms of action of THC including reduced CD8+ T cells, increased lamina propria FoxP3+ T regulatory cells, increased colonic barrier integrity and increased mucous deposition and tight junction production.

X-linked inhibitor of apoptosis protein (XIAP) deficiency is a rare monogenetic condition often associated with refractory IBD[29]. Successful allogeneic haematopoietic stem cell transplantation (HCT) is curative[30]. In addition to inducing endoscopic and clinical remission, Ono et al[31] demonstrated that HCT in 19 patients with XIAP deficiency and IBD was shown to improve gut dysbiosis using 16S rRNA sequencing of faecal samples. However, improvement in alpha diversity independent of reduced inflammatory burden was not demonstrated.

Blockade of the pro-inflammatory catalysing enzyme phosphodiesterase-4 (PDE4) downregulates IFN-γ, TNF and IL-17 and promotes synthesis of IL-10[32,33]. Whilst used in a range of pulmonary and dermatological disorders, PDE4 inhibitors have not yet been established in UC and are subject to early phase clinical trials of apremilast[34]. In a murine model of DSS-induced colitis, mice receiving apremilast for 30 days *via* oral gavage had attenuated clinical, macroscopic and histological disease activity and maintained intestinal mucosal integrity compared to controls[35]. Furthermore, mice receiving apremilast had greater relative abundance of beneficial microorganisms, such as Lactobacillus and Bifidobacterium and reduced pro-inflammatory microbes using 16S rRNA sequencing. Passive faecal transfer from control mice to apremilast-treated mice by co-housing contributed to a worsening in both symptom severity and mucosal inflammation, suggesting a putative role for the dysbiosis.

Finally, there were advancements in the selective manipulation of gut microbiota as an adjunctive therapy in IBD. FimH is a potent gut TLR4 agonist which can trigger lipopolysaccharide-independent inflammatory cascades[36]. Chevalier et al[37] targeted bacterial adhesion of FimH-expressing adherent-invasive *E. coli*. Relative to healthy controls, shotgun metagenomic sequencing of faecal samples from 358 patients with CD demonstrated greater relative abundance of *Enterobacteriaceae spp*, including *E. coli*, alongside a direct association with clinical disease activity. Secondly, on ileal biopsies of 106 CD patients, 65% of patients displayed adherent colonies made up of FimH-expressing bacteria, all restricted to *Enterobacteriaceae* spp. Utilising human intestinal explants, FimH blockade by TAK-018 reduced bacterial adhesion to intestinal epithelium and subsequently down regulated inflammation and loss of mucosal integrity. TAK-018 offers an attractive, orally administered and gut selective avenue for manipulation of pathogenic microbial machinery and requires prospective evaluation in an upcoming phase II study in post-operative Crohn's disease (NCT03943446).

FAECAL MICROBIOTA TRANSPLANT

Faecal microbiota transplant (FMT) remains a novel and emerging strategy in contrast to conventional IBD therapeutics. FMT however, is relatively well-established in the treatment of refractory or recurrent *C. difficile* infections. Transplantation of the faecal microbiota aims to reintroduce and establish a 'healthy' community of microbes which are known to be disrupted (microbial dysbiosis) in the gut of IBD patients. One current research focus is the establishment of faecal banking and administration protocols that optimise the efficacy and safety of FMT in the treatment of IBD.

Nicholson et al[38] build on existing efficacy in *C. difficile* treatment in a paediatric IBD cohort (CD 45%, UC 49%, IBD-U 6%). IBD diagnosis did not significantly impact the efficacy of FMT (76% IBD, 81% non-IBD) in treating recurrent *C. difficile* infection (rCDI). Whilst IBD activity was not the primary aim of this study, it was found that of the FMT responders 38% had an improvement in IBD status. Conversely, failed FMT was linked to clinically active IBD or a post-FMT disease flare. Three factors were associated with greater FMT success: fresh stool transplant, non-diarrhoeal host stool consistency and shorter time from *C. difficile* infection onset to treatment. In a separate cohort, examining the impact of FMT on paediatric UC, a rigorous schedule of 12 FMT enemas resulted in 92% of patients reaching the composite clinical endpoint (improvement in paediatric UC activity index, C-reactive protein, or faecal calprotectin)[39]. However, clinical remission (Pediatric Ulcerative Colitis Activity Index <15) rates were lower with 33.3% and 41.7% of UC patients in clinical remission at week 6 and week 30, respectively. This pilot randomised controlled trial did not reach primary feasibility outcomes as the recruitment target was not reached. Pai et al[39] highlight recruitment complexities in both paediatric studies as well as therapeutic interventions. Twelve enemas over a 6-week period are an intensive schedule and potentially more frequent than required.

In a small adult cohort (n=8), similar methodology was applied to assess FMT-based treatment of rCDI in IBD patients[40]. Unlike the aforementioned studies, donors were instructed to consume magnesium hydroxide (oxidative laxative) prior to faecal collection. Participants also underwent bowel purge with polyethylene glycol prior to the administration of fresh stool *via* colonoscopy. Faecal microbiota composition of the participants was assessed before FMT as well as 2 and 8 weeks post. Beta diversity between patients and donors was not significantly different post-FMT. Whilst an increase of *Bacteroidetes* was observed over the 8 weeks, there was no significant difference in alpha diversity[40]. *Prevotella* was the single genus found to significantly increase over the course of the study. Metabolic pathway analysis revealed oxidative phosphorylation to be low in the pre-FMT samples with higher gene abundance post-FMT. This study resulted in rCDI symptomatic resolution in all 8 participants. Despite the lack of a control or placebo group, the study design incorporated microbial and functional analysis to show FMT impact. *Prevotella* appeared to be crucial to the success of FMT. Whilst it was highlighted that patients did not adopt or inherit the microbial composition of donors; it was hypothesised that FMT allowed restoration of their own healthy microbiome[40].

Lima et al[41] performed donor strain sorting and sequencing of immunoglobulin A coated microbiota to highlight immune-reactive components of FMT. The clinical endpoint (Mayo score ≤3) was reached in 35% of participants. Key transferable microbiota strains were then identified and compared between donor and recipient as well as responders and non-responders. A unique set of 12 donor-derived strains were identified to correlate a clinical response to FMT. *Odoribacter splanchnicus* was identified as a key species active component of the FMT. Functional assessment shows anti-inflammatory action (IL-10 and lymphocyte-dependent regulation) as well as healthy metabolite production (SCFA producer), highlighting the importance of quality donor material.

Selecting an appropriate and safe donor sample is key in FMT efficacy. Zhang et al[42] explored donor screening procedures in a Japanese population. Their methods consisted of general health questionnaires pre-screening, formal laboratory screening of blood and stool and final screening to re-confirm health status at time of enrolment. In the 2014-2018 cohort, 50% passed the donor screening process compared to 25.6% in the 2018-2021 cohort. Of the excluded volunteers in both cohorts, most were screened out at the preliminary assessment phase (health background) with only 4 (n=138) and (n=117) volunteers excluded due to

pathogen prevalence in blood or stool in the 2014-2018 and 2018-2021 cohorts respectively. The authors highlight the importance of rigorous donor screening to maintain the safety of FMT even when associated cost and volunteer exclusion can be high. This study also highlighted that whilst patients may prefer a known donor this may not be the safest option.

Donor and autologous FMT were compared in the treatment of chronic antibiotic-dependent pouchitis[43]. Participants (n=26) received two FMTs on week 2 and two FMTs on week 4 of the study. The first dose was administered *via* endoscopy with subsequent doses *via* a transanal catheter. To improve treatment retention, patients were also administered 2 mg loperamide. Relapse occurred in 9/26 donor FMT recipients, 4 before the second FMT dose. Only 8 autologous FMT participants relapsed and none within the first 4 weeks of the study. Improved quality of life was reported by FMT group participants at the 26 week follow up. Antibiotic dependence was also selected for within the study criteria which may have impacted uptake and efficacy of the FMT[43].

The impact of donor dietary manipulation was explored by Sarbagili Shabat et al[44]. Three groups of active UC patients received enema FMT. Free diet donor (group 1) and controlled diet donor with dietary intervention on patient (group 2) were compared to a dietary intervention only group (group 3; no FMT). FMT was administered *via* enema with a dose of loperamide. FMT efficacy was low in both groups (Group 1: 11%, Group 2: 21%). Faecal samples from a single donor induced remission in 60% of recipients, suggesting a 'super donor' effect. Donor microbiome analysis confirmed the dietary intervention period did not significantly alter composition. The trial was suspended at 53% recruitment due to futility. The impact of dietary fibre administered alongside FMT was explored by Clancy et al[45], however, the efficacy of FMT was not assessed directly. Observational symptoms were reported in relation to dietary intake. This pilot study also included irritable bowel syndrome (IBS) alongside patients with IBD. In the IBD group, anti-inflammatory medication and antibiotic pre-treatment was also used to induce remission. No significant trends were found in relation to fibre intake, gastrointestinal symptoms and quality of life in this study.

Zhao et al[46] aimed to explore risk factors for clinical recurrence of IBD post FMT. A cohort of 192 patients (UC and CD) were enrolled. Fifteen patients had symptomatic recurrence within the first week post-FMT (11 UC, 4 CD). In UC patients, Mayo endoscopic sub-item score (MES), C-reactive protein (CRP), anaemia, albumin, absolute peripheral blood lymphocytes (PBL) and intolerance to enteral nutrition were found to be risk factors of disease flare during and post FMT. Hypoalbuminaemia and simultaneous use of immunosuppressive agents were associated with recurrence of disease in CD patients during and post FMT treatment. Březina et al[47] compared FMT to 5-ASA treatment in a cohort (n=43) with active left sided UC. Patients were recruited with clinical Mayo scores of 4-10 and randomly assigned 1:1 to receive FMT enemas or 5-ASA enemas. 16S Ion Torrent sequencing was used to assess the microbial profile in faecal samples collected at 2, 4, 6, and 12 weeks. Both treatments resulted in an increase in microbial diversity which was sustained longer in the FMT group. Higher microbial diversity and positive treatment outcomes correlated with higher diversity of donor stool. Treatment success was achieved in 12 of 21 FMT patients and 8 of 22 5-ASA patients. These studies demonstrate a comparable safety profile to existing therapeutics.

Ren et al[48] describe results from a single centre historical control trial. Two doses of FMT were administered with an interval of 2 months. UC patients underwent a one-week washout period prior to study commencement. Polyethylene glycol was used for bowel preparation prior to fresh donor FMT administration *via* colonoscopy (with or without gastroscopy). Clinical and endoscopic remission was achieved in 22.58 % of patients after the first FMT and 60 % after the second. At the 4-year follow-up, 4 of 12 patients that achieved early remission remained in remission. Alpha diversity was initially lower but rose to donor levels post-FMT. The samples were initially enriched in *Bacteroides fragilis, Clostridium difficile,* and *Ruminococcus gnavus* but the abundance of these species was decreased post-FMT. Comparing only the patients which achieved remission, *Bifidobacterium breve* was higher in those which sustained long-term remission status. These results show promising efficacy for low intensity FMT administration.

FMT was similarly shown to be effective in a higher intensity yet novel protocol using patient prepared stool samples. In this study, corticosteroid-dependent UC patients underwent three sessions of FMT *via* colonoscopy at fortnightly intervals[49]. The FMT solution was self-pre-

pared by the participant from a chosen volunteer donor. Bowel preparation with polyethylene glycol was used as well as 4 mg loperamide after FMT administration. By week 4, 48% of participants achieved clinical remission and tapered steroid use. 48% at week 24 achieved steroid and azathioprine-free clinical remission, with 76.9% showing a histological response to the FMT treatment. However, the majority of patients (76.9%) experienced relapse before the 24 week follow up. This was the first published study including home preparation of FMT solution. However, administration was still performed in a clinical setting. These results are promising for home preparation and highlight the importance of maintenance FMT, suggesting 6-monthly intervals may be required[49].

The use of oral FMT was explored by Haifer et al[50] in a double-blind placebo-controlled trial in patients with active UC. The oral FMT consisted of 0.35 g of lyophilized stool. Two-week pre-clearance with antibiotics (amoxicillin 500 mg three times daily, doxycycline 100 mg twice daily, and metronidazole 400 mg twice daily) was followed by a regimen of six FMT capsules administered four times per day for one week, followed by six FMT capsules twice per day for six weeks. Corticosteroid-free clinical remission was achieved in 73% of FMT patients *vs.* 15% in the placebo group ($p = 0.005$). Corticosteroid-free endoscopic remission was seen in 47% of FMT *vs.* 15% of placebo group participants ($p = 0.06$). Comparing microbial profiles, FMT significantly increased microbial richness compared to the placebo treatment. Compositional change was noted as early as week 1, with saturation in diversity occurring at week 3. An increase in donor prevalent species was also observed with no significant difference between responders and non-responders[50]. Patients in the placebo group who responded at week 8 showed a linear rather than an exponential post-antibiotic increase in species richness. Again, a strong donor effect was observed in FMT-treated patients, with a higher success rate from donor stool dominant in Bacteroides and enriched with *Prevotella*. This intensive study of orally administered FMT demonstrated similar comparative efficacy to FMT administration *via* colonoscopy or enema[50].

Two studies focussed on patient perceptions of FMT. Qualitative interview data was collected from a cohort of paediatric patients who had undergone a treatment regimen of bi-weekly FMT enema for 6 weeks[51]. An adult cohort of patients who consented and patients who declined FMT were interviewed by Chauhan et al[52] to understand patient perspectives of FMT. Both cohorts discussed the impact of social stigma and the "yuck factor" of FMT impacted willing-ness and perceptions of the treatment[51,52]. Pre-treatment, both cohorts also expressed concerns over potential pain or physical discomfort as both studies assessed enema administered FMT patients. A perception of "naturality" was also mentioned by both groups. In the Chauhan cohort those who did not partake in FMT posed this as scepticism to alternative medicines. Most patients reported positive experiences post FMT. Despite this, lack of FMT accessibility was a large disadvantage. Parents of paediatric patients and adult FMT patients seemed to have limited understandings of the FMT procedure and the end objectives of the procedure. Overall, FMT was well received by patients but could benefit from better point of care education[51,52].

DIET AND PREBIOTICS

Dietary interventions, pre- and probiotics are of growing community and scientific interest as a therapeutic option for IBD patients. Several research groups have studied the relationship between these interventions and the gut microbiome in IBD in the last year.

Bolte et al[53] discovered some interesting associations between dietary patterns and the gut microbiome in UC, CD and IBS patients as well as in healthy volunteers. More specifically, they found that animal foods, processed foods, alcohol and sugar correlated with an inflammatory profile, characterised by a higher abundance of Firmicutes, *Ruminococcus* species of the *Blautia* genus and an upregulation of endotoxin synthesis pathways. On the other hand, plant-based foods and fish associated with anti-inflammatory features such as an increase in short-chain fatty acid-producing commensals, microbial metabolism of polysaccharides and a decrease in pathobionts.

Similar results were seen by another research group where a Mediterranean diet affected the microbiota composition with an increase in bacteria with anti-inflammatory

features (e.g., Actinobacteria, Verrucomicrobia such as *Akkermansia)* and a decrease in taxa with pro-inflammatory properties (e.g., Fusobacteria such as *Fusobacterium*, Proteobacteria, *Ruminococcus* genus from Lachnospiraceae). The opposite was observed in IBD patients[54].

In a cross-over randomized controlled trial, the effects of a low-fat, high-fibre diet (LFD) vs. an improved standard American diet (iSAD, included higher quantities of fruits, vegetables, and fibre than a typical SAD) was studied in 17 UC patients in remission. Interestingly, LFD significantly lowered inflammation markers (serum amyloid A and serum CRP) and reduced the intestinal dysbiosis in faecal samples characterized by a decrease in Actinobacteria, an increase in Bacteroidetes and F. *prausnitzii*, increased faecal levels of acetate and tryptophan and decreased levels of lauric acid[55].

In a single-arm, pre-post intervention trial, 25 IBD patients were subjected to an IBD-Anti-Inflammatory Diet (IBD-AID), with an increased intake of monounsaturated and polyunsaturated omega-3 and fatty acids, pre- and probiotics while eliminating saturated fats, trans-fatty acids and processed foods, as previously described by Peter et al[56]. Consumption of the IBD-AID diet correlated with an increased abundance of the SCFA-producing genera Clostridia and Bacteroides, which are often depleted in IBD patients. Furthermore, butyrate-producing species (*Roseburia hominis* and *F. prausnitzii*) and acetate-producing species (*Eubacterium eligens* and *Bacteroides dorei*) were augmented. Moreover, high intake of prebiotics and beneficial foods were found to be associated with lower levels of the cytokines IL-6 and IL-8 and higher levels of GM-CSF[57].

A study in 307 healthy men found that the intake of dietary fibre (mostly driven by pectin and fruit fibre) resulted in an altered gut microbiota composition with an increase in Clostridiales such as *F. prausnitzii* and *Eubacterium eligens* and a decrease in *Lachnospiraceae* and *Ruminococcus*. In this population, inflammation-associated gut microbiome compositions could be linked to higher CRP levels. Moreover, fibre intake was associated with a greater CRP reduction in individuals without the presence of *Prevotella copri* in the gut[58]. In this regard, fibre supplementation may reflect a potential dietary treatment option for IBD patients. However, a separate *in vitro* experiment using faecal samples revealed that IBD patients in remission had a similar capacity to ferment fibre and release SCFA compared to healthy controls, despite the presence of a microbial dysbiosis in the IBD group. Therefore, fibre supplementation alone might not be enough to lower the inflammatory burden in patients with IBD[59].

When taking a closer look at the effect of a combination therapy in IBD patients, Xiang et al[60] investigated the effect of the combination of exclusive enteral nutrition (EEN) and a washed microbiota transplantation (WMT) – performed immediately (on day 1) or delayed (on day 8) in a randomized open-label study in 19 patients with CD complicated by malnutrition. They showed that EEN combined with immediate WMT more rapidly improved nutritional parameters and resulted in a higher rate of clinical remission compared to WMT alone.

Taken together, the studies described above suggest that a healthy diet such as a Mediterranean diet, a low-fat high-fibre diet, an IBD-AID and a diet with plant-based foods and fish has the potential to shift the microbiota composition towards an anti-inflammatory phenotype. The effect on both disease onset and clinical trajectory requires further investigation. Finally, the observation of an interaction effect between a diet, the gut microbiome and a systemic marker of inflammation (CRP) introduces the potential for a more personalised diet-based approach in the management of IBD[58].

Besides the effect of dietary therapy, two separate studies investigated the effect of a prebiotic intervention in IBD. The effect of prebiotic galactooligosaccharide (GOS) supplementation was tested in 17 patients with active UC. Whilst no effects were observed on clinical scores or inflammation, stool consistency was normalised. Moreover, the relative abundance of *Bifidobacterium* and *Christensenellaceae* was increased in patients with less active disease (simple clinical colitis activity index SCCAI ≤2). This may indicate that the beneficial prebiotic effect may be dependent on disease activity[61]. Another study examined the effect of a 3-week treatment with the prebiotic oligofructose/inulin (15 g/day) in 19 patients with inactive CD and 12 healthy siblings. After 3 weeks, no change in calprotectin levels was observed. Interestingly, prebiotic treatment had greater impact in

healthy siblings compared to inactive CD patients. Faecal Bifidobacteria and *Bifidobacterium longum* was increased in patients and siblings, while *Bifidobacterium adolescentis* and *Roseburia spp*. was only increased in siblings. Intestinal permeability decreased significantly in IBD patients after oligofructose/inulin to a level that was similar to siblings however circulating T cell abundance was reduced in siblings but not IBD patients after oligofructose/inulin treatment[62].

PAEDIATRICS

Several studies investigated whether composition of the gut microbiome, mycobiome and microbial metabolites can be utilised in the diagnosis and prediction of disease phenotypes in paediatric IBD.

In line with previous studies in an adult population, Putignani and colleagues found that the faecal microbiota composition of a paediatric IBD population had a higher abundance of bacteria with pro-inflammatory characteristics (e.g., Proteobacteria, Actinobacteria, *Haemophilus parainfluenzae*) and a lower abundance of bacteria with anti-inflammatory properties (e.g., Bacteroidetes, Tenericutes, Verrucomicrobia *Ruminococcus* and *Oscillospira*). Furthermore, Fusobacterium was found to be increased in inflamed colonic mucosa samples compared to non-inflamed and control samples[63].

Another study was able to stratify paediatric CD patients with dysbiosis from paediatric CD without dysbiosis and healthy controls based on their metabolic profile. Moreover, in IBD, the reduced microbial diversity resulted in a lower number of secreted metabolites, especially those related to sulfur metabolism, and an increased potential to synthesise amino acids, which was linked to Proteobacteria[64].

Breton et al[65] found that children with perianal fistulising CD have a unique microbial signature with a reduced butyrogenic profile, which is distinct from patients with CD without a perianal phenotype and healthy controls. Whether this signature has a causative role in the development and persistence of perianal CD requires further evaluation.

Finally, the investigation of fungi and archaea profiles in faecal samples from children with active CD, non-active CD and healthy controls revealed an increased prevalence of *Candida tropicalis* in active CD compared to non-active CD and controls. In addition, patients with active CD had lower abundance and patients with inactive CD had a higher abundance of *Malassezia* spp. compared to healthy paediatric controls. No statistical significant differences between the investigated groups were observed for the archeobiome[66].

In an attempt to improve diagnostic capacity in paediatric IBD, a machine learning model integrating the top 11 operational taxonomic units (OTUs), from a total of 1902 OTUs was able to successfully differentiate between IBD and controls and therefore could be an interesting avenue for the development of a non-invasive tool in the diagnosis of pre-clinical paediatric IBD[67]. In addition to the diagnostic potential, it may also reflect a potential interesting treatment target in this population. More specifically, after a longitudinal analysis of both the microbiota and the inflammatory status in a paediatric UC population that received an anti-inflammatory treatment during 1 year, it was suggested that combination therapies, targeting both inflammation and the microbiota would improve the treatment efficacy in that population[68].

Finally, two separate studies explored the effect of EEN in paediatric CD patients. In the first study, the effect of EEN was tested for 8 weeks in 31 newly diagnosed paediatric CD patients. After EEN for 8 weeks, a range of clinical and objective parameters (disease activity index, FCP, weight gain, haemoglobin, serum albumin, endoscopic severity score) were significantly improved in two-thirds of patients and bacterial diversity was increased[69]. The second study investigated the impact of EEN on the microbiome in paediatric CD patients and healthy siblings. They found that changes in microbiota diversity occurred faster in healthy siblings (at day 2 of EEN) compared to CD patients (at the end of EEN, after 6 weeks). At the end of the EEN course, the change in microbiota composition of children with CD was comparable to that of healthy siblings, with a significant increase in Firmicutes and a tendency for a decrease in Proteobacteria[70].

MYCOBIOME

The role of gut mycobiota in human health and disease, including IBD, remains under preliminary investigation. In the previous year, only four articles were chosen for review. In a publication in *Nature Microbiology*, Doron and colleagues demonstrated that, in addition to its role in modulating resident microbiota, intestinal secretory IgA can also regulate fungal commensalism[71]. In particular, *C. albicans* was shown to induce plasma cell IgA class switching and subsequent IgA antibodies showed affinity for hyphae-associated virulence factors in an associated murine model. In mucosal washings from patients with CD, flow cytometry-based analyses revealed an increase in granular fungal morphologies and reduced *C. albicans*-induced sIgA targeting hyphae-associated virulence factors. Together, these data suggest a dysregulated antibody response against pathogenic hyphal morphotypes in CD. The significance in the pathogenesis and disease course of CD requires further evaluation.

In a later publication, the same group provided early mechanistic insight into gut host-fungal crosstalk. Li et al[72] demonstrated that patients with UC had a consistent increase in *C. albicans* compared with individuals without IBD both in colonic lavage samples and mucosa confirmed by a culture-dependent approach. However, introduction of *C. albicans* did not exacerbate the severity of DSS-induced murine colitis nor did it precipitate spontaneous colitis following prolonged colonisation. Using culture-based methods, the authors also demonstrated that cell damage and pro-inflammatory stimulation by human-gut-derived *C. albicans* are strain dependent and linked to filamentation ability and the production of IL-1β, perhaps highlighting an adjunctive therapeutic avenue in the management of UC.

In a separate human observational study, Nelson et al[73] evaluated the impact of NOD2 gene mutations on the gut microbiota in patients with CD and healthy controls. Whilst patients with CD in remission were shown to have higher fungal diversity and a higher abundance of *Candida* spp. than healthy controls, there was no difference in the faecal mycobiota between NOD2 wild-type or mutant individuals with or without CD. Similarly, Sharifinejad and colleagues evaluated the association between leucine-rich repeat kinase 2 (LRRK2) SNPs, a predisposing genetic mutation for IBD, and faecal yeast species in patients with UC and healthy controls[74]. *Candida* spp. were again found in greater abundance amongst UC patients when compared to healthy controls, independent of LRRK2 SNP. Together, these studies indicate that the increased susceptibility to CD and UC conferred by NOD2 mutations and LRRK2 SNP, respectively, are unlikely to be mediated by alterations in the mycobiome.

CONCLUSIONS

This review presents the highlights in a year of important advances in the investigation of the relationship between gut microbiota and IBD. A further understanding of the modulation of gut microbiota by conventional IBD therapeutics and FMT, the impact of a range of dietary therapies and the exciting potential of targeted, microbiota-specific manipulation to impact disease course are of particular interest. The significance of the mycobiome and the role of gut microbiota in paediatric cohorts remains under preliminary investigation. We have also presented a number of developments in the underlying genetics and pathophysiology driving host-microbiota interactions in IBD. However, future research that interrogates the multidirectional cross-talk between host immune cells, components of the intestinal barrier, microbial diversity and metabolite expression may uncover clearer aetiological pathways and allow identification of novel therapeutic avenues for IBD.

Conflict of Interest

RDL has received educational support from Janssen. WA, SK and HC have no relevant conflicts of interest to declare.

Acknowledgements

We would like to acknowledge the supervision and guidance of Professor Georgina Hold and Professor Annemieke Smet.

Authors' Contribution

All authors have made substantial contributions to the conception and design of the literature review, screening and selection of articles, drafting the article and revising it critically for important intellectual content. All authors have provided final approval of the submitted manuscript.

Funding

No external funding was required or received for this review. HC would like to acknowledge general funding support from the Research Foundation Flanders (FWO) no. 1244421N (junior postdoc).

Consent

No additional consent is required for a review of published scientific literature.

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