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Microbiota and IBD

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Abstract: The current article is a review of the most important, accessible, and relevant literature published between April 2018 and March 2019 on the gut microbiota and inflammatory bowel disease (IBD). The major areas of publications during the time period were in the areas of human clinical studies as well as mechanistic insights from colitis models. Most papers focused on the bacterial component of the gut microbiota although some papers described aspects of the virome and mycobiome. Paediatric IBD focussed studies are the focus of a separate review within this series and are therefore not included here. Over 86 relevant papers were published in the reporting period.

Keywords: Inflammatory bowel disease, Microbiome, Microbiota, Colitis models.

IBD MODELS/MECHANISTIC PAPERS

Gut microbiota shapes and it is shaped by the host immune system, through reciprocal development, maturation, and regulation. In one of the more popular models of spontaneous chronic colitis, the IL10$^{-/-}$ mouse model, gut microbiome dysbiosis occurs before the development of spontaneous colitis in mice as a result of IL10 signaling disruption. The same model does not develop colitis under germ-free conditions, suggesting that immunity and microbiota are interdependently relevant for disease development[1]. However, the complexity of intestinal mucosal ecology can induce unpredictable interactions. A recent paper gave light to Phosphatase and tensin homolog (Pten), a phosphatase protein produced by a tumor suppressor gene, whose activity has been associated with greater mucosal damage in the IL10$^{-/-}$ colitis model[2] and also susceptibility to ambient pollutants. The intestinal application of Pten-specific inhibitor VO-OHpic leads to a more severe colitis in the absence of IL10, which was also associated with an increase in colitogenic bacteria, in particular, *Bacteroides* and *Akkermansia*. The study also showed that in the presence of IL10 Pten-specific inhibition by VO-OHpic was not associated with the development of colitis. The cross-talk between immunity and microbiota affected by Pten is, therefore, crucially relevant for IL10$^{-/-}$ associated colitis. Another important finding suggested by this paper was that in pro-inflammatory conditions, the species *Akkermansia* can act as a colitogenic species, similar to *Bacteroides* species[3,4]. The role of Pten in mediating microbe-induced immune responses in the gut was also reinforced by Howe et al[5], who, using IEC-specific Pten knockout mice (Pten$^{\Delta IEC/\Delta IEC}$), demonstrated a significant decrease in fecal *Akkermansia muciniphila* levels in Pten$^{\Delta IEC/\Delta IEC}$ mice which conferred a tumour-preventative intestinal environment.

Other murine models of colitis that were studied during the reporting period included SOCS1$^{-/-}$ in which SOCS1 expression was restored in T and B cells (SOCS1$^{-/-}$Tg mice), Nod2$^{-/-}$ mice and mice carrying the Crohn's disease polymorphism, ATG16L1 T300A. Although SOCS1 is part of a negative feedback loop to attenuate cytokine signaling, in this model of spontaneous chronic colitis, a dysbiosis was described with an increase in *Prevotella*, *Bilophila*, and *Streptococcus*[6]. The dysbiosis induced an increase in TNFalpha but not of IL-1 beta and IL6,

suggesting an IκB zeta-independent process. In Nod2$^{-/-}$ mice, although depletion of this gene was not associated to spontaneous colitis, neonatal antibiotic treatment significantly and permanently changed the gut microbiota composition with a higher susceptibility to severe colitis than controls[7]. In mice carrying the Crohn's disease polymorphism, ATG16L1 T300A, the gut microbiota was altered (mainly the increase in the order *Bacteroidales*, including *Bacteroides ovatus*) and associated with an enhanced intestinal Th1/ Th17 response leading to a heightened DSS colitis susceptibility[8].

Studies showed, however, that targeting innate/adaptive immunity can also induce a "protective" dysbiosis, able to prevent or attenuate colitis. Nunberg et al[9] described the dysbiosis of IL1α-deficient mice conferring protection against DSS-induced colitis. The microbiota of IL1α KO mice was characterized by lower relative abundances of *Coriobacteriaceae*, *Bacteroides* and *Akkermansia,* both before and after exposure to DSS compared to untreated mice. An abundance of *A. muciniphila,* an anaerobic, mucus-degrading bacterium considered protective for active colitis was associated with an increase in severity of colitis, a finding which contrasts with other models of colitis. Cohousing of IL-1 KO with WT mice diminished their resistance to DSS induced colitis. In microRNA-21 (miR-21)$^{-/-}$ mice, a reduced susceptibility to DSS-induced colitis was associated with an increased proportion of *Bifidobacteriaceae* and *Peptostreptococcaceae* as well as a reduction in *Clostridium XIVa* species *Tannerella, Dorea and Bacteroidetes* members belonging to *Barnesiella* and *Prevotella,* relative to WT mice[10]. This protective phenotype was also transferrable to germ-free (GF) mice colonized with fecal homogenate from miR-21$^{-/}$ mice, compared with WT homogenate.

Intestinal inflammation can also be modulated by extra-intestinal intestinal chronic inflammation, such as psoriasis. In a recent paper, TLR-dependent dermatitis, induced by Imiquimod in a murine model of psoriasis, was associated with exacerbated DSS colitis in mice which was thought to result from an altered immune cell composition in the intestine which followed the onset of dysbiosis[11]. In particular, topical application of Imiquimod (necessary for the psoriasis model), directly decreased B cells in the gut in a microbe-independent manner with a concomitant increase in the number of macrophages was also detected. Microbiota analysis indicated the application of Imiquimod was associated with a decrease in *Lactobacillaceae* and *Desulfovibronaceae*.

Other important concepts reinforced by recent studies relate to the dysbiotic effect of some drugs, including infliximab (anti-TNFα), or diet. Petito et al[12] assessed microbiota changes in healthy mice exposed to infliximab and showed that increased levels of *Enterococcaceae* along with a decrease in *Bacteroides* and *Clostridiaceae* were present following infliximab treatment. This observation is particularly relevant if translated in humans: the potential dysbiotic effect of immunogenic drugs could represent a novel therapeutic target in order to maximize clinical remission and response to drugs.

VIROME/MYCOBIOME/ARCHAEA

The majority of studies looking at understanding the role of the gut microbiota in IBD focus on bacteria, however other microorganisms including viruses, fungi and archaebacteria are present in significant numbers[13]. For example, DNA and RNA viruses that collectively make up the intestinal virome are at least equivalent in number to bacterial cells, although on gut mucosal surfaces and within the mucus layers they may significantly outnumber bacterial cells. The human GI tract virome community is predominated by prokaryotic viruses known as bacteriophages (phages). Phage numbers in the intestine, which exceed 10^{15} bacteriophage particles, are known to increase during IBD although to date there is a limited understanding of their role in disease pathogenesis. Duerkop et al[14] used a T cell adoptive transfer mouse model (Rag $1^{-/-}$ injected with $CD4^{+}$ $CD45RB^{High}$ T cells) to study intestinal phage dynamics during colitis. They observed a decrease in phage community richness alongside an expansion of phage subsets in mice with colitis. The phage lineages that expanded were frequently connected with bacterial hosts that are known to induce intestinal inflammation including Enterobacteriaceae and Enterococci. In addition, expansion of *Caudovirales* family members including *Siphoviridae, Myoviridae* and *Podoviridae* were noted in T-cell treated animals, findings which correlate with previous human IBD patient studies[15]. Additionally, Firmicutes

specific phages including phage that infect Lachnospiraceae, Ruminococcaceae and *Clostridia* were identified alongside phages specific for Streptococci and Bacteroidetes, specific to *Alistipes* even though 16S rRNA gene read abundances did not demonstrate an expansion of these bacteria in mice with colitis. The findings also demonstrated a significant overlap in phage community structure between murine and human hosts highlighting the suitability of mouse models for assessing phage/bacterial interactions in the context of human IBD. A human study, published in Gut, characterised the human gut virome in UC patients[16]. Deep metagenomic sequencing of virus-like particle preparations and bacterial 16S rRNA sequencing were performed on the rectal mucosa of 167 subjects from three different geographical regions in China (UC=91; healthy controls=76). An increase in *Caudovirales* bacteriophage numbers was detected in UC patients although *Caudovirales* species diversity and richness actually reduced compared to healthy subjects. *Escherichia* phage and *Enterobacteria* phage were also more abundant in UC patient mucosal samples compared to healthy subjects.

Further investigation of the role of the virome was undertaken by Ungaro et al[17], to profile the eukaryotic virome in young treatment-naive patients from the RISK study. Reporting on comprehensive metagenomic analysis of gut mucosae they found *Hepadnaviridae* were highly abundant in ulcerative colitis (UC) patients whilst *Hepeviridae,* which includes *Hepatitis E* virus*,* was seen in high abundance in Crohn's disease (CD) patients. Other viral families including a number of viruses usually seen to infect plants and insects, namely *Polydnaviridae* and *Tymoviridae,* were less abundant in UC, whilst *Virgaviridae* was less abundant in CD.

As well as a growing interest in the virome, there has been a growing appreciation for the role of fungi, referred to as the mycobiome, in IBD pathogenesis. The mycobiome has recently been shown to be altered (increased Basidiomycota/Ascomycota ratio) in IBD[18,19] as well as IBD patients developing increased titers of anti-fungal antibodies. However, fungal:bacterial interactions and the impact of antibiotic treatment on gut fungal loads in IBD has not been explored. Using a dextran sodium sulphate (DSS)-induced colitis model, Sovran et al[20], evaluated the effect of daily administration of *Candida albicans* and *Saccharomyces boulardii. C. albicans* is the most prevalent fungal species detected in the human gut and is able to thrive in the presence of gut inflammation, whilst *S. boulardii* has been shown to protect against IBD symptoms in previous murine colitis models. In the current study, *C. albicans* administration caused increased colitis severity whilst *S. boulardii* was shown to have the opposite effect, actually reducing colitis symptoms. To further interrogate how the commensal bacterial community modulated the effects of these fungal strains, the experiment was repeated following 7-day broad spectrum antibiotic treatment prior to colitis induction. Antibiotic treatment protected the mice from the impact of fungal strains. Elucidation of bacterial community structure changes in response to antibiotic treatment showed that *Enterobacteriaceae* levels were significantly reduced, suggesting that potentially loss of these species may protect the host against the development of colitis. Using different specific antibiotics (Vancomycin – targeting Gram-positive organisms, and colistin – targeting *Enterobacteriaceae*), the authors were able to demonstrate that Gram-positive organisms most likely belonging to the families *Ruminococcaceae* and *Lachnospiraceae* are required to trigger DSS-induced colitis whilst the presence of *Enterobacteriaceae* was required for *C. albicans* and *S. boulardii* species to exert their negative and positive respective effects.

Further exploration of the mechanisms associated with fungal involvement in IBD pathogenesis were reported by Malik et al[21]. Previous findings have shown that fungi are potent inducers of the multimeric inflammasome protein complexes that act as sensors for a variety of pathogen-associated molecular patterns (PAMPs). Mutations in genes encoding components of the inflammasome complex as well as fungal recognition genes are associated with IBD[22-24]. Despite this knowledge, there is little understanding of how fungal-sensing pathways including CARD9 and the upstream activator spleen tyrosine kinase (SYK) impact on inflammasome activation. Malik et al, demonstrated that commensal gut fungi, that signal via the SyK-CARD9 pathway, protect against experimental colitis and that SYK and CARD9 are required for inflammasome activation by both *Candida* and *Aspergillus* species. Limon et al[25] showed that the presence of the skin resident fungus *Malassezia restricta* was associated with the colonic mucosa in CD patients and its presence was linked to the presence of an IBD-associated polymorphism in CARD9. *M. restricta* exacerbated colitis via CARD9 in mouse models of disease and enhanced inflammatory cytokine production from innate cells containing the IBD associated CARD9 polymorphism.

The prevalence of *Methanobrevibacter smithii*, a methanogen producing archaea group species, was assessed in an Iranian IBD patient cohort and compared with healthy controls[26]. Using qPCR analysis, they showed that when assessed collectively (all IBD patients whether in remission or having active disease) IBD patient faecal samples had a significantly decreased *Mbb. smithii* load compared to healthy controls. When stratified by disease activity (remission *vs.* active disease), higher levels of *Mbb. smithii* were seen in remission. Previous studies looking at the relationship between *Mbb. smithii* abundance and IBD have demonstrated inconsistent findings. Further work to establish the role of archaea bacteria in IBD pathogenesis is required.

HUMAN STUDIES

In IBD, disease severity and treatment response are strikingly heterogeneous. The molecular mechanisms driving this variability represent a critical knowledge gap that needs to be addressed in order to formulate targeted therapeutic approaches. Haberman and colleagues used RNAseq to define pre-treatment rectal mucosal gene expression, and fecal microbiota profiles, in 206 pediatric UC patients[27]. They observed upregulated gene signatures, enriched for integrin signalling, JAK-STAT cascade components, and TNF production; pathways that are already the target of therapeutic strategies in UC. They also reported the suppression of mitochondrial genes in active UC, and that increasing disease severity was aligned with enrichment of adenoma/adenocarcinoma and innate immune genes. A subset of these genes could be used to improve prediction of corticosteroid-induced remission in the discovery cohort. The gene signature, which included Oncostatim M and TREM1, was also associated with response to anti-TNFα and anti-$\alpha4\beta7$ integrin in adults. The results may prioritise future therapies for non-responders to current approaches.

IBD microbial signatures vary across populations according to ethnicity and geography as well as in response to disease activity. In a small Chinese cohort, Ma et al reported a greater abundance of Proteobacteria in IBD patient stool samples compared to healthy controls, which is consistent with existing literature[28,29]. However, they also documented the increased abundance of *Haemophilus* and decreased *Desulfovibrio* in UC stool, a finding not previously reported in Chinese patients[30], adding to the growing literature in this traditionally low-prevalence group.

Heidarian and colleagues assessed the abundance of key bacterial families in stool samples from a small group of IBD patients (UC n=22, CD n=7) compared to healthy controls (n=29)[31]. All samples were transferred to the laboratory within one hour of collection for DNA extraction. They noted that *Bacteroides, Faecalibacterium prausnitzii, Prevotella* spp., and *Methanobrevibacterium* were significantly less abundant in IBD patients, especially those with active disease. Higher levels of *Streptococcus* and *Haemophilus* were noted in active IBD. To determine microbial mechanisms in disease pathogenesis, the authors co-cultured HT-29 cells with donor stool. They discovered that exposure to IBD stool, especially samples with abundant Enterobacteriaceae, led to increased IL-18 induction. Mirsepasi-Lauridsen and colleagues analysed the fecal microbiome in a sizable group of 25 healthy controls and 97 IBD patients with both active and inactive UC and CD, including 18 patients with an ileal pouch[32]. Lower Shannon Diversity Index was seen in CD and pouch patients with active disease compared to the inactive stage. In UC patients, a generally lower diversity was observed at all stages of the disease compared to healthy controls.

Irritable bowel syndrome (IBS) is a common diagnosis that affects up to 20% of the global population and frequently co-exists with IBD, leading to significant morbidity. Two studies investigated the fecal microbiome signature of IBS. Shutkever et al[33] studied a cohort of 270 IBD patients stratifying them into four groups: IBS-type symptoms, quiescent disease, occult inflammation, and active disease. Apart from a non-significant increase in the abundance of Actinobacteria in the stool of patients, reporting IBS-type symptoms, there were no distinct microbiota signatures. In a larger study, Vila and colleagues performed shotgun metagenomic sequencing of stool samples from 1792 individuals with IBD and IBS and compared the results with population controls[34]. Unlike Shutkever, they differentiated between IBS diagnosed by a gastroenterologist (IBS-GE) from patients reporting IBS-like symptoms in a questionnaire (IBS-POP). They also inferred bacterial growth rates from the pattern of sequencing read

coverage [peak-to- trough ratio (PTR)] across the gut bacterial genomes single metagenomic sample. Whilst no microbial shifts were noted in the stool of IBS-POP cohort, IBS-GE displayed decreases in several butyrate-producing bacteria, including *Faecalibacterium prausnitzii* and *Roseburia intestinalis*, similar to IBD patients in the study. IBS-GE was also associated with an increase in several *Streptococcus* species. The authors also reported 1) increased growth rates of *B. fragilis* and *Escherichia coli* amongst CD patients; 2) a pro-inflammatory metabolic environment in CD characterised by decreased fermentation pathways, higher sugar degradation, and increased quinone biosynthesis. In UC, butyrate and acetate pathways were diminished, whereas in IBS-GE metabolic signatures were characterized by increased fermentation and carbohydrate degradation pathways. An increased abundance of bacterial virulence factors amongst individuals with IBD and IBS-GE were also reported including increased adhesion and immune evasion.

While IBD has been associated with dramatic changes in the gut microbiota, changes in the gut metabolome, the molecular interface between host and microbiota, are less well understood and is the increasing focus of IBD research. Using metabolomic and shotgun metagenomic profiling of stool samples from discovery and validation cohorts of IBD (68 CD and 53 UC patients) and healthy control subjects (n=34), Franzosa and colleagues identified chemicals and chemical classes that were differentially-abundant in IBD, including enrichments for sphingolipids and bile acids, and depletions for triacylglycerols and tetrapyrroles[35]. In total, they identified 122 robust associations between differentially abundant species and metabolites, indicating possible mechanistic relationships that are perturbed in IBD. They concluded that metabolome- and metagenome-based classifiers of IBD status were highly accurate and may serve as potential diagnostic and therapeutic targets.

LONGITUDINAL STUDIES

Longitudinal data in microbiome studies remain scarce. It is unclear what the frequency of sampling should be, but the Academy Colloquium on "Delivering the most effective treatment to every patient with inflammatory bowel disease" held on April 2018 recommended sampling every 3 months as a minimal requirement[36]. This meeting also emphasised the need for well-powered longitudinal studies in specific contexts such as fecal microbiota transplantation (FMT). The meeting was necessitated by the emergence of multiple microbiome consortia throughout the world and their efforts to create large datasets comprising extensive clinical and microbiome data. The goal of the meeting was to integrate a worldwide collaborative effort to leverage data for two key objectives: 1. prioritize new targets for drug development and 2. Discovery of biomarkers to deliver personalized medicine. It highlighted key knowledge gaps including how the microbiota can be used for treatment response prediction, the need for consensus on FMT delivery, and the need for mechanistic studies to define the role of specific IBD-associated microbial species and derived metabolic compounds.

In the last year, the most promising prospectively-established IBD microbiome databank is the 1000IBD project, which has enrolled 1215 participants at the time of writing[37]. 1000IBD project is collecting clinical, diet, environmental, genome, transcriptome, and microbiome data with the collection of prospective clinical data incorporated into the regular IBD management, serving both as electronic health record and a research dataset. At the time of writing, over 500 stool samples have been collected, with stool samples frozen within 15 minutes of sample donation. The 1000IBD data is a versatile resource which future researchers investigating IBD can utilise as a replication cohort or as a pilot cohort to test new hypotheses.

Yilmaz et al[38] described microbial networks related to long-term disease severity and responsiveness to different treatment modalities. Analysing mucosal biopsies from a large cohort of IBD patients and healthy controls. Over 5000 mucosal biopsies had been collected longitudinally as a part of the Swiss IBD Cohort Study. Healthy controls all had normal findings on screening colonoscopy and normal blood tests including inflammatory markers. Biopsies taken throughout the disease course over periods ranging up to 9 years, were analysed to define microbiota composition. They found that generally individual taxa were generally stable over time, even with inter-current fluctuations in disease activity. Only Enterobacteriaceae and *Klebsiella* in CD patients and *Ruminococcus* and *Prevotella* in UC showed

consistent compositional changes between the cohorts that were significantly aligned with clinical assessments of disease activity. These findings indicate that microbiota composition profiles were mainly personalised rather than disease activity-specific. To further define critical variables in shaping individual microbiota, the authors used the algorithms in the hierarchical all-against-all association (HAllA) tool for multi-resolution associations. They identified BMI and age as the most important variables correlating with gut microbiota of IBD patients. To a lesser extent, disease type, location and behaviour and prior surgery were also important variables showing interactions with more than 60-70% of most abundant taxa of IBD patients. Of lifestyles factors correlating with microbiota profile, smoking was the most significant. A major burden for IBD patients is the relapsing-remitting disease course. The Yilmaz study showed that in CD, *Eggerthella, Clostridiales* and *Oscillospira* abundance showed consistent replicated increases in relative abundance in patients with quiescent disease over time while *Enterobacteriaceae* and *Klebsiella* were associated with a more severe clinical course. *Oscillospira* abundance also was associated with a more benign course of CD.

Disease therapy including monoclonal antibody treatment against TNF-α and steroids are utilised with variable degrees of efficacy in IBD patients. Yilmaz and colleagues also aimed to determine whether the responsiveness of IBD patients to different therapies could correlate with variability in microbiota composition. Amongst CD patients receiving TNF-α inhibitors, increased levels of *Bifidobacterium*, *Collinsella*, *Lachnospira, Roseburia, Eggerthella* taxa and reduced *Phascolarctobacterium* were associated with treatment success. No significant trends were seen in UC cohorts or amongst IBD patients receiving corticosteroid therapy.

A few smaller longitudinal IBD microbiota studies were also published in the timeframe; however, these were small and did not sample at regular intervals. In a small longitudinal study of CD patients, Galazzo et al[39] did not detect shifts in faecal microbial richness or diversity at baseline or during disease exacerbation. Kiely et al longitudinally sampled mucosal microbiome in IBD patients and noted considerable fluctuation over time[40]. However, the greatest changes occurred in the presence of ongoing intestinal inflammation. Samples from patients with previous abdominal surgery had lower alpha diversity. Walujkar et al[41] investigated the changes in mucosal microbiota amongst Indian UC cohort during acute exacerbations and subsequent remission. They reported increased abundance of *Stenotrophomonas, Parabacteroides, Elizabethkingia, Pseudomonas, Micrococcus, Ochrobactrum* and *Achromobacter* during exacerbation compared to remission phase.

OUTSIDE OF THE STOOL – MUCOSAL AND MLN MICROBIOTA STUDIES

The faecal microbiota is distinct from the mucosal microbiota, with the latter believed to better represent the inflammatory and immunological microenvironment of this mucosal disease. Traditionally stool samples are more easily obtained that mucosal samples and were also easier to derive microbial community data from. Bacterial numbers are lower in mucosa samples and as sequencing technologies have improved, both in terms of sensitivity but also cost, there has been a shift in emphasis towards studying the intestinal mucosal microbiota and, beyond this, microbes in the draining mesenteric lymph nodes which may play a central role in driving systemic inflammation. Al-bayati et al[42] isolated bacteria from colonoscopic biopsies of inflamed mucosa and rectum of a small cohort of patients with and without UC, respectively. As noted in previous studies, they found a decrease in *Faecalibacterium prausnitzii, Prevotella spp.,* and *Peptostreptococcus productus*. Altomare and colleagues reported an increase in Proteobacteria (especially *Enterobacteriaceae*, *Acidaminococcus*, *Veillonella dispar*) and a decrease of Firmicutes in the mucosal microbiota of IBD compared to controls, which was not seen in matched faecal samples, suggesting the microbiota adhering to the gut mucosa better discriminates patients from controls[43]. The study was too small to determine any associations between mucosal disease activity and microbial shifts. Chiodini et al[44] analysed microbiota from mucosa and submucosa of resected terminal ileum tissue from UC and CD patients. Interestingly, the normal appearing tissue at the resection margins showed evidence of bacterial translocation, with two bacterial families, *Comamonadaceae* and *Xanthomonadaceae* having penetrated the mucosal surface.

Akkermansia muciniphila and *Faecalibacterium prausnitzii* are considered members of a healthy microbiota and reduction of both species occurs in several intestinal disorders, including inflammatory bowel disease. To investigate a possible link between their reduction and disease states, Lopez-Siles et al[45] measured their abundance in colonic biopsies from 17 healthy controls, 23 patients UC and 31 patients with CD. They noted reduced numbers of *F. prausnitzii* in subjects with CD, compared with healthy controls. Depletion of *A. muciniphila* was only noted in paediatric onset CD, potentiating its utility as a biomarker to assist diagnosis. *Yersinia* are common contaminants of food products and have been implicated in CD. Le Baut et al[46] analysed a large number of ileal biopsies from CD patients and controls; however, *Yersinia* were no more likely to be detected in CD tissues than controls.

Using the transcribed 16S rRNA counts, as opposed to the gene counts, to indicate the potential bacterial metabolic activity of a sample, Moen et al[47] investigated the composition of active mucosal microbiota of a small group of treatment-naïve UC patients to identify active microbial members of the microbiota in early stages of the disease. Several taxa, belonging to the Proteobacteria phyla revealed lower transcriptional activity in inflamed samples despite being present in higher numbers compared to uninflamed samples. Members of the *Bifidobacteriaceae* family also showed lower levels of transcriptional activity in the active microbiota, but in contrast to the Proteobacteria, there was no difference in abundance detected.

Mesenteric lymph nodes are sites in which translocated bacteria incite and progress immunological responses. For this reason, understanding the microbiome of mesenteric lymph nodes (MLNs) in inflammatory bowel disease is important. Kiely et al[48] demonstrated that MLNs from IBD patients had greater alpha diversity than in paired mucosal biopsies, reflecting their larger drainage area. A second study assessed microbial diversity in MLNs from 5 CD and 8 UC patients undergoing intestinal resection[49]. They noted that CD MLNs contained fewer Firmicutes and higher Proteobacteria compared with UC samples. Although species diversity was reduced in MLNs from patients with Crohn's disease, the nodes contained greater numbers of less dominant phyla, including Fusobacteria which is known to play a role in the pathogenesis of UC[50]. Determining the pathogenic mechanisms associated with the presence of Fusobacteria in MLNs should be the focus of future research.

Tye et al[51] demonstrated that increases in NLRP1, the inflammasome sensor, within inflamed colonic mucosa of UC patients is associated with increased IFN-γ. In this context, NLRP1 or IFN-γ expression negatively correlates with the abundance of beneficial, butyrate-producing Clostridiales in human rectal mucosal biopsies. They were further able to negate the colitis-protective effects of Nlrp1 deficiency using vancomycin and reproduce it once more with butyrate supplementation in wild-type mice. These findings indicate the NLRP1 inflammasome may be a key negative regulator of protective, butyrate-producing commensals, which therefore promotes IBD.

GENETICS/MICROBIOTA

To date, over 240 IBD risk genetic loci have been identified, many of which are involved in microbial handling/recognition[52]. Whilst IBD is thought to be caused by an inappropriate host immune response to the gut microbiota, the precise nature of this interaction remains elusive despite the increasing number of mechanistic and observational studies which have investigated the interplay between host genetic variation and the gut microbiota. A study by Aschard et al[53], published in PLoS Genetics, used observational data to explore the relationship between genetic variants in four major genes associated with increased IBD risk (*NOD2, CARD9, LRRK2,* and *ATG16L1*) and specific components of the gut microbiota. Using linear regression modelling and replication analysis, they were able to show that IBD risk alleles in *NOD2* were negatively associated with the abundance of genus *Roseburia* as well as *Faecalibacterium prausnitzii.* The *NOD2*/*F. prausnitzii* finding was confirmed using qPCR analysis in the discovery cohort as well as 3 replication cohorts from the USA and the Netherlands comprising 451 IBD subjects. A second study presented an integrative analysis of CD related genetic risk loci (*NOD2, ATG16L1, IRGM, CARD9, XBP1,* and *ORMDL3*) and ileal microbiota composition in a combined cohort of 293 patients comprising 1) ileal CD patients undergoing ileocolic resection, 2) IBD colitis patients without ileal disease (UC, CD, and indeterminate

colitis), and 3) non-IBD subjects. Proteobacteria abundance was positively associated with ileal CD but surprisingly negatively associated with *NOD2* risk loci. The study also indicated that IBD medications such as immunomodulators and anti-TNF medications may help restore the dysbiosis associated with the disease phenotype. The exploratory analysis also highlighted the potential of microbial biomarkers from resected tissue to predict post-surgical disease recurrence risk. A smaller study from Australia assessed the impact of *NOD2, ATG16L1* and *IL23R* genetic polymorphisms on the gut microbiota in mucosal samples (paired ileal and rectal biopsies) from 15 ileal CD patients and 58 healthy subjects[54]. Only the IL23R R381Q mutation had an impact on intestinal microbiota composition with healthy subjects carrying the protective allele having an increased abundance of *Christensenellaceae, Bacteroides caccae* and *Oscillospira*.

Genetic risk loci associated with IBD have also been detected in protein tyrosine phosphatase non-receptor type 2 (PTPN2) and PTPN22. PTPN2 is involved in maintaining epithelial barrier function, limiting pro-inflammatory cytokine secretion as well as directing T cell differentiation and function. Single nucleotide polymorphisms in PTPN2 are associated with an excessive pro-inflammatory phenotype. PTPN22 is involved with regulation of the NLRP3 inflammasome through the control of tyrosine phosphorylation. Based on their role in host:microbial interactions, Yilmaz et al, investigated the influence of PTPN2 and PTPN22 gene variants on intestinal microbiota composition and disease course in IBD patients[55]. Using mucosal biopsy samples from the Swiss IBD cohort study, they included a cohort of 142 IBD patients (75 CD: 57 UC). In CD patients carrying PTPN2 risk loci, higher levels of unassigned genera belonging to *Clostridiales* and *Lachnospiraceae* families. In UC patients, a reduction in *Roseburia* was seen in patients carrying PTPN2 risk loci compared to PTPN2 wildtype patients, with an increase in *Ruminococcus* also seen in UC patients carrying PTPN22 risk loci. The study also showed differences in intestinal microbiota composition in patients with severe disease course and carrying PTPN risk loci although these findings were based on a low number of patient samples.

Epigenetic factors, defined as factors which mediate interactions between the environment and the genome are also thought to play a central role in IBD pathogenesis and other diseases. One of the main epigenetic mechanisms includes RNA interference; transmitted by microRNAs (miRNAs). miRNAs have been explored as biomarkers and therapeutic targets, since they are able to regulate specific genes associated with CD. In a small cohort of CD patients, Rojas-Feria and colleagues reported three miRNAs were induced in affected mucosa: mir-144, mir-519 and mir-211, which have not been described before, and may serve as potential biomarkers[65].

INTERVENTIONS/THERAPEUTICS

The potential of nutritional interventions, enrichment with bioactives/supplements, or re-seeding of the microbiota including probiotics, continue to dominate IBD research papers. Three separate studies[57-59] looked at the impact of polyphenol supplementation on the intestinal microbiota in murine models of colitis. Both Lin et al[57] and Hong et al[59] used a *Citrobacter rodentium* colitis model to assess the effect of quercetin supplementation. Quercetin was shown to reduce colitis severity through reduction of pro-inflammatory cytokine levels. In addition, quercetin supplementation increased levels of *Bacteroides*, *Bifidobacterium*, *Lactobacillus,* and *Clostridia* and significantly reduced *Fusobacterium* and *Enterococcus* numbers[57]. Liso et al[58] described the effect of a 2-week bronze tomato diet enriched with polyphenols including quercetin. The 2-week diet was sufficient to cause changes to gut microbiota composition in mice, however, changes were murine strain-dependent.

Iron deficiency anaemia is a significant extraintestinal manifestation for IBD patients. Whilst iron is a key nutrient for pathogenic bacteria, little is known about the impact of altered iron on microbiota associated with IBD, with the best way to administer iron replacement to patients being a subject of intense debate. Mahalhal et al[60] investigated the effect of differing dietary iron levels on DSS induced colitis. Excess dietary iron caused a significant reduction in the abundance of *Firmicutes* and *Bacteroidetes*, and an increase in *Proteobacteria.* The role of vitamin D as a potential immunomodulator in patients with IBD has been investigated extensively for over a decade. Studies have shown that vitamin D plays a role in

the regulation of the epithelial barrier, innate immune cell and T-cell function, as well as preliminary data suggesting that vitamin D may influence the intestinal microbiota in IBD. Garg et al[61] investigated the effect of vitamin D supplementation at a dose of 40000 IU weekly for 8 weeks in IBD patients. Vitamin D supplementation caused a reduction in circulating and intestinal markers of inflammation in patients with active UC as well as a significant increase in abundance of *Enterobacteriaceae* in patients, and a trend to reduction in the mucolytic species *Ruminococcus gnavus*, although overall microbiota diversity was unchanged. A second small prospective, longitudinal, controlled interventional study investigated the effect of orally administered vitamin D on intestinal microbiota composition in CD[62]. Vitamin D caused a temporal shift in microbiota composition in CD patients although a further increase in vitamin D level was associated with a reversal of this effect and additionally with a decrease in the bacterial richness in the CD microbiota. A further study by Ghaly et al[63], investigated the effect of high dose vitamin D supplementation in an acute DSS colitis model. Vitamin D supplementation led to a more severe DSS-induced colitis as well as microbiota changes including increased levels of *Sutterella* and Bacteroidales S24-7 group members.

Probiotics which correct bacterial imbalances play an important role in the treatment of IBD. A number of publications assessing the effect of probiotic strains on gut microbiota profiles in chemical-induced colitis models were published during the year; the effect of *Lactobacillus plantarum* and *Escherichia coli* Nissle 1917 were studied within DSS models of colitis, whilst *Bifidobacterium longum* and VSL#3 were assessed in a TNBS model[64-66]. Both *L. plantarum* and *Escherichia coli* Nissle 1917 treatment reduced disease activity scores as well as increasing colonic microbial diversity and Firmicutes/Bacteroidetes ratio but interestingly *L. plantarum* supplementation reduced the relative abundance of *Lactobacillus*[64,65]. Other bacterial strains including various *Bacteroides* strains demonstrate anti-inflammatory properties in DSS models of colitis. Previous studies have shown that the human commensal *Bacteroides fragilis* ameliorated colitis severity in a T-cell transfer or TNBS models. Lee et al[67] assessed its impact within the DSS model. They demonstrated that *B. fragilis* was able to suppress the development of intestinal inflammation via inhibition of CCR5 expression. The protective effects of *Bacteroides thetaiotaomicron* were also assessed within various rodent colitis models by Delday et al[68]. *B. thetaiotaomicron* showed protective effects in both DSS and IL10 knockout rodent models. These effects were not exclusive to actively growing bacterial preparations but were retained by freeze-dried cells of *B. thetaiotaomicron*.

Dietary interventions including palmatine, goji berries, quinoa and berberine were all investigated in the context of animal models of colitis whilst human intervention studies assessed the impact of inulin-type fructans and cobalamins. Palmatine treatment significantly abrogated DSS-induced colitis and facilitated restoration of intestinal homeostasis including gut microbiota community structure[69]. Compared to DSS treatment alone, palmatine treated mice has substantially recovered bacterial populations including Bacteroides, Lactobacillus and Ruminococcus whilst numbers of pathogenic bacteria including Proteobacteria and Desulfovibrio were reduced. The prebiotic effects of the South American edible grain quinoa were also evaluated in a DSS colitis model[70]. Quinoa is rich in high-quality protein, vitamins and minerals, and contains various polysaccharides which have immune-regulating activity in animal studies. Similar to Palmatine, quinoa supplementation significantly reduced disease activity indices and suppressed the expansion of *Proteobacteria* of the genera *Escherichia/Shigella* and *Peptoclostridium* seen following DSS treatment.

Assessment of the herbal medicine formulation *Codonopsis pilosula* (Franch) Nannf (CPN), which contains large amounts of saponin and polysaccharides, and is widely used to replenish Qi (vital energy) deficiency and strengthen the immune system, was also studied using the DSS-induced colitis mouse model[71]. CPN showed prebiotic-like effects enhancing the abundance of 3 species – a *Bifidobacterium* spp., *Lactobacillus* spp., and an *Akkermansia* spp., whilst inhibiting the growth of pathogenic bacteria, including *Desulfovibrio* spp., *Alistipes* spp., and *Helicobacter* spp. Microbiota abundance changes were accompanied by increased short chain fatty acid levels, upregulated the expression of anti-inflammatory cytokines and downregulated the secretion of proinflammatory cytokines correlated with Th17/Treg balance. Goji berry supplementation increased *Actinobacteria, Lachnospiraceae* and *Ruminococcaceae* but decreased *Peptostreptococcaceae* and no effect on *Bacteroides, Akkermansia, Mucispirillum* and *Desulfovibrio* in IL10$^{-/-}$ mice[72]. Singh et al[73] sought to examine the effects of refined fibres on coli-

tis development. Dietary pectin protected against the development of αIL-10R-induced colitis, whilst being associated with a reduction in *Verrucomicrobia,* which encompasses *Akkermansia muciniphila* and an increase in the short chain fatty acid-acetate*,* while inulin promoted colitis development in an NLRP3-dependent manner and was associated with an increase in gamma-*Proteobacteria* as well as butyrate. Excess butyrate levels correlated with a worsening of colitis scores which were improved by targeted suppression of fermentation activity. The study highlighted that not all fermentable fibres have the same effects and a greater understanding in the context of personalised fibre-based interventions is required.

An exploratory human pilot study compared the clinical effects of inulin-type fructans in mild to moderately active UC[74]. Nine weeks of supplementation (15 g daily) led to limited microbial compositional changes (increased *Bifidobacteriaceae* and *Lachnospiraceae* abundance) although this did not correlate with improved disease scores. More importantly, colonic butyrate levels were increased and negatively correlated with Mayo score (UC activity score). Other studies[75,76] also assessed the impact of vegetarian or gluten-free diets and fruit consumption on the IBD microbiota. Using the Swiss IBD Cohort Study, Schreiner et al[75] demonstrated that specific diets, i.e. vegetarian or gluten-free (self-reported), are associated with microbiota changes but these do not impact on disease course; however, specific diets correlated with lower psychological well-being compared with omnivores.

Emerging diagnostic markers of an imbalanced gut-microbiota-host interaction have also been proposed. In particular, a fecal miRNA profile was demonstrated to differentially and specifically stratify by microbiota composition, predicting the colitogenic potential of the microbiota[77]. Faecal miRNA profiles were compared between germ-free and conventional mice with colitogenic and non-colitogenic microbiotas ($IL10^{-/-}$ and $TLR5^{-/-}$ associated microbiota). Correlation analysis revealed that 12 miRNAs were identified that were impacted by the presence of a microbiota and that miRNA could importantly serve as markers of the colitogenic potential of the microbiota. Germ-free and conventional mice with a colitogenic and non-colitogenic microbiotas showed altered fecal miRNA profiles compared to that of with a "healthy" microbiota. The findings could be particularly relevant to study individual microbiota profiles in patients with IBS as well as other diseases characterized by dysbiosis.

Conflict of Interests:

The Authors declare that they have no conflict of interests.

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