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# YEAR IN MICROBIOTA: IBD MECHANISTIC STUDIES

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**Abstract –** The review explores one year of the most important, accessible and relevant original scientific publications published between April 2020 and March 2021 exploring the microbiome of infants, children and adolescents. This review encompasses 40 studies describing changes in microbiota composition observed in paediatrics in a wide spectrum of pathologies in addition to the development of the microbiome during infancy and early childhood and the impact of nutritional intervention and antibiotics on the gut microbiome.

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

## DIETARY IMPACT IN IBD – EVIDENCE FROM PRECLINICAL STUDIES

The role of diet and dietary components in the pathogenesis, prevention, progression, and remission of colitis is a central area of current IBD research. The role of diet on inducing or preventing inflammatory response through various mechanisms, such as gene regulation, microbial regulation, and inflammatory pathways are being explored. Most dietary studies are conducted using modified murine models to replicate states of colitis as seen in IBD.

A randomised controlled trial utilised an IL-10 knockout mice model to explore the impact of pomegranate extract supplementation (PomX) on colitis development and progression[1]. Mice were fed a high fat high sucrose (HFHS) diet with or without PomX for 8 weeks. Compared to the control group, mice fed with supplement revealed prominent decreased incidence in rectal prolapse, improved inflammatory histological scores, significantly lower serum interleukins, regulation of genes associated with inflammatory response and neutrophil degranulation, and an increase in microbiota alpha diversity scores with no alteration in beta diversity measures. An increased abundance of Verrucomicrobia with a simultaneous decrease in Epsilonbacteraeota and Actinobacteria in PomX fed mice was seen. The results revealed that PomX exhibited an overall anti-inflammatory effect which resulted in reduced inflammatory markers and improved colitis scores.

Other studies were conducted using dextran sulphate sodium (DSS) induced acute colitis murine models to explore the effects of various nutrients on IBD pathogenesis. The effect of rice protein peptides (RPP) on this model was explored by Yang et al[2]. The study demonstrated that treatment with RPP alleviated inflammatory symptoms such as significant decrease in weight loss, colon shortening, lowered disease activity index (DAI) score, improved regulation of inflammatory pathways and anti-inflammatory gene expression. The study also found that RPP increased Akkermansia abundance which was known to be an important short chain fatty

acids producing bacterium. The impact of ceramide compared to glucosylceramide (GlcCer) also depicted similar results to suppression of decreased body weight[3]. However, dietary ceramide improved the DAI scores more than GlcCer. It was also noted that ceramide consumption resulted in decreased proinflammatory gene expression, thereby inhibiting neutrophil infiltration and reducing inflammation. The supplementation also resulted in restoration of bacterial abundances which were increased or decreased by DSS. Bacteroidetes and Firmicutes levels were maintained with ceramide while they were decreased by DSS. γ-Proteobacteria, which was increased by DSS, was reduced by ceramide and/or GlcCer. Further, an independent study conducted in China explored the impact of bioactive substance Oxymatrine (OMT)[4]. The results were similar to other studies that used the same model wherein the DAI scores improved along with reduced symptoms of colitis. Moreover, OMT was found to decrease dendritic cell numbers involved in pro-inflammatory pathways with a simultaneous regulation of the enteric microbiome. OMT administration after DSS seemed to correct the dysbiosis caused through an increase in *Muribaculacear* and *Ruminococcaceae* with simultaneous decrease in *Staphylococcaceae, Bacteroidaceae* and *Prevotellaceae*. The effect of another compound, oryzanl (ORY) was also explored by using the same mouse model[5]. The findings also showed a significant improvement in colitis symptoms which was regulated by the inhibition of inflammatory gene expression and upregulation of anti-inflammatory gene expression. Alterations in gut microbiota profiles were also observed with ORY increasing abundance of *Alloprevotella, Roseburia, Treponema, Muribaculaceae,* and *Ruminococcus* genera which are known to contain several SCFA producing species.

Extract of oolong tea and betaine supplementation were also found to alleviate inflammatory responses through downregulation of proinflammatory cytokines and upregulation of anti-inflammatory cytokines in the DSS model[6,7]. It was also shown that the tea extract reduced pathobiont abundance (Escherichia and Shigella) while promoting levels of *Lachnospiraceae* and therefore exerting an overall protective effect against intestinal inflammation. In contrast, betaine altered gut microbiota profiles with a decrease in Firmicutes and Proteobacteria alongside an increase in Bacteriodota and Campylobacterota. Mulberry extract (MAS) was also proven to be effective against DSS induced colitis[8]. MAS was shown to suppress colonic oxidative stress while restoring protein expression to uphold the tight junction integrity. Like other supplements, it also reduced DSS induced dysbiosis with decreased Escherichia-Shigella, increased *Akkermansia, Muribaculaceae* and *Allobaculum* resulting in improvements in colitis scores.

A *Citrobacter rodentium* (CR)-induced colitis model was also utilised to examine the effect of barley leaf (BL)[9]. BL was shown to improve inflammatory scores with lowered DAI scores and colitis symptoms. Similarly to other dietary compounds, BL was also found to impact gut microbiota profiles with an increase in Lactobacillus and a decrease in Proteobacteria. However, unlike the other dietary components, BL was shown to be effective more so as a pre-treatment and therefore could be used as a prevention strategy.

One study looked at the microbial strains found in a traditional Chinese fermented food, jiang-shui, and whether the strains exerted a protective factor against IBD pathogenesis[10]. They isolated 38 lactic acid producing strains and tested their effects using the DSS model. Their study found one strain, *Lactobacillus plantarum* JS19 exerted similar effects in alleviating colitis as seen in other DSS model studies with reduced DAI scores, inhibition of weight loss and amelioration of colon damage. The strain was found to improve inflammation through reducing lipid peroxidation and inhibition of TNF expression along with other pro-inflammatory pathways. It also reverted the gut dysbiosis caused by DSS.

A randomised controlled study evaluating the impact of maternal diet on the offspring gut microbiome and colitis development showcased that a maternal high protein diet had a significant impact on gut microbial diversity and abundance whereas high fat diet did not impact the microbial population to the same extent[11]. Offspring from each group were then divided into two groups; one would continue the maternal diet and one converted to control diet. Offspring who continued a HFD after birth had increased weight gain and a higher disease activity index (DAI) score after DSS challenge compared to control diet counterparts. In contrast, offspring from maternal high protein diet group presented with a lower body mass, significant decrease in colon length with DSS, and a significantly greater DAI scores regardless of continuation or alteration of their diet. A greater dysbiosis was also prominent in the HFD group.

Another study using a mouse model using germ free Tnf ΔARE mice and wild-type (WT) mice looked at the impact of diet on Segmented Filamentous Bacteria (SFB) as they have been associated with severity of IBD[12]. The murine model used here has been shown to develop intestinal inflammation resembling the transmural ileal disease phenotype as seen in CD. Their results depicted that the presence of SFB significantly impacted the severity of colitis developed in the mice. It was also elucidated that a purified diet inhibited the development of intestinal inflammation in the mice as compared to chow diet. The levels of SFB were significantly lower or not detected in those treated with purified diet (PD). Mice under purified fibre-rich diet (FRD) also emitted similar results to PD group.

There were variations in TNF-α expression levels which correlated with SFB colonisation and diet group. SFB was seen growing close to intestinal epithelium of mice on chow diet and to a lesser extent in the FRD group. There was no visible SFB growth near the epithelium on PD diet-fed mice suggesting that diet played a crucial role in SFB colonisation and disease pathogenesis.

A systematic review and meta-analysis of preclinical studies investigating the potential therapeutic effects of licorice in UC was published during the reporting period[13]. Lu et al[13] identified 27 relevant studies and found that licorice and its bioactive compounds, such as glycyrrhizin and liquiritin, have anti-inflammatory, antioxidant, and immunomodulatory effects that may be beneficial in treating UC. The study also used network pharmacology to identify potential targets and pathways involved in the therapeutic effects of licorice in UC. The results suggest that licorice may exert its therapeutic effects through multiple mechanisms, including regulating cytokines, oxidative stress, and the gut microbiome. The study highlights the potential of licorice as a natural product for treating UC. It provides a basis for further research in this area but cautions that the potential non-reporting of negative findings and variability in study design and quality must be addressed.

Pi et al[14] found that alginate (ALG) – a naturally occurring biodegradable polymer extracted from marine brown algae and commonly used as an additive in food, medicines and cosmetics industries alleviated DSS-induced colitis. While the reparatory effects of ALG in the context of IBD have been previously established, this study suggests that the mechanism involves selective enrichment of the probiotic *Bifidobacterium animalis* and hyodeoxycholic acid (HDCA). HDCA arises from converting the primary bile acid b-muricholic acid (bMCA) via the multistep 7a-dehydroxlyase pathway in various intestinal bacteria. Pi et al[14] observed a reciprocal change in HDCA and bMCA in response to ALG – a significant increase in the former and suppression of the latter – in mice colonic digesta, which was additionally correlated with a threefold increase in *B. animalis*, making the species the most abundant in the colon (21.5%). Their work confirmed previous findings, including a strong correlation between HDCA and Clostridium sp. and the downregulation of pro-inflammatory cytokines interleukin (IL)-1β, IL-6 and TNF-α in the mouse colon.

Chen et al[15] investigated the effect of tea polysaccharides (TPS) and tea polyphenols (TPP), both individually and in combination (TPS + TPP), in a DSS-induced mouse model of colitis. TPS and TPP separately administered significantly reduced the relative abundance of *Enterobacteriaceae*. No such effect was observed in the group receiving TPS + TPP, which instead showed enrichment of *Lactobacillaceae*. When comparing the three treatment combinations, they found that the best reparatory effects on DSS-induced body weight, disease activity index, colon length and colonic tissue damage were attributed to the TPS + TPP treatment group. Likewise, the most remarkable improvement was observed in this group when assessing mucin and tight junction protein expression.

The correlation between intestinal barrier repair in DSS-induced mouse colitis and nutritional therapy appears consistently across the papers included in this section. Specifically: royal jelly[16], mannose[17], spermidine[18], and the dietary oligopeptide Ile-Arg-Trp[19], in addition to the above TPS/TPP. Royal jelly (RJ) – a milk-like substance secreted by worker bees for the nourishment of larvae and the colony's queen – was assessed for its therapeutic potential in a DSS-induced mouse model of colitis[16]. The most beneficial effect was observed in the highest dosage group (2.0 g/kg) which resulted in a significant decrease in intestinal permeability which also correlated with an increase in mucin and tight junction protein expression. This treatment group also showed an increased level of anti-inflammatory cytokines IL-10 and sIgA within colonic tissue. An increase in the relative abundance of *Erysipelotrichaceae* of the Fir-

micutes phyla, in conjunction with a decrease in typically inflammatory constituents, including *Parabacteroides* and *Proteobacteria*, was also observed in the 2.0 g/kg RJ group compared to the untreated DSS group.

A similar reparatory effect on tight junction protein expression was observed by Dong et al[17] in DSS-induced mice treated with mannose, a monosaccharide involved in protein glycosylation in mammals. They found that mannose supplementation specifically disrupted the phosphorylation of myosin light chain two, which is established in the disruption of the tight junction barrier. In combination with the first-line IBD treatment mesalazine, the therapeutic effect was enhanced in the mouse model.

Niechcial et al[18] assessed the therapeutic capacity of the naturally available polyamine spermidine, found in ribosomes and living tissue, in a T cell transfer colitis model in Rag2-/- mice. Their results demonstrate that spermidine treatment ameliorated intestinal inflammation. However, the efficacy of spermidine hinged on the presence of the anti-inflammatory molecule protein tyrosine phosphatase non-receptor type 2 (PTPN2) in intestinal epithelial cells and myeloid cells. This finding is consistent with existing research on spermidine's potency in PTPN2 activation and confirmed that this receptor's upregulation increased the expression of anti-inflammatory M2 macrophages. Beta diversity analysis of microbial communities (using principal coordinates of analysis) in mouse faeces revealed that spermidine-treated colitis mice and healthy controls clustered closer than the untreated disease group, suggesting spermidine's potential to ameliorate intestinal microbial dysbiosis.

Ile-Arg-Trp (IRW), a peptide derived from egg ovotransferrin with a demonstrable anti-inflammatory effect, was the focus of an investigation by Fei et al[19] in the context of intestinal injury and microbial impact using a DSS-induced colitis mouse model. IRW intervention (0.04 mg/mL group specifically) significantly inhibited the depletion of goblet cells and therefore maintained mucus layer integrity compared to the untreated DSS mice group. Their microbial analyses suggest that IRW can remodel the gut microbiota by increasing the relative abundance of Firmicutes and *Lactobacillus* and suppressing the abundance of Bacteroides. IRW also regulated the relative abundance of *Odoribacter.* Fei et al[19] also found that IRW treatment significantly increased SCFA content in mouse faeces.

Plasmon-activated water (PAW) has been found to have anti-inflammatory properties. Chang et al[20] examined the effects of PAW on colonic immune activity and microbiota in mice with IBD induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS)[21]. PAW consumption was found to mitigate IBD symptoms and increase the abundance of *Akkermansia muciniphila*, a potential next generation probiotic species[22]. Furthermore, the study revealed that TNBS-induced IBD reduced the abundance of *Akkermansia* spp. in the gut, while PAW consumption restored levels. PAW also increased the microbial diversity and abundance of beneficial species including *Akkermansia* spp., *Roseburia* spp., and *Oscillibacter* spp., while reducing the abundance of *Marvinbryantia* spp. Additionally, another study showed that *A. muciniphila* played a protective role in the colon by regulating immune responses and maintaining gut homeostasis. TLR4-deficient mice exhibited enhanced susceptibility to colitis and a reduced proportion of suppressive RORγt+ Treg cells, while the administration of *A. muciniphila* mitigated colitis in both wild-type and TLR4-deficient mice[23]. The findings suggest that *A. muciniphila* and PAW have beneficial effects on colonic cells and can potentially alleviate IBD symptoms by modulating the gut microbiota and immune responses.

## PROBIOTICS AND IBD – EVIDENCE FROM PRECLINICAL STUDIES

Several strains of Lactobacillus have shown potential in relieving colitis symptoms and have been the focus of numerous IBD studies. Studies conducted in the period of interest demonstrated that the potential is strain specific with *Lactobacillus acidophilus* CCFM137 and FAHWH11L56 in improving colitis symptoms on DSS-Induced Colitis models, while L. acidophilus FGSYC48L79 did not provide protection. *L. acidophilus* NCFM and FAHWH11L56 had similar effects, increasing levels of the anti-inflammatory cytokines IL-10 and IL-17, as well as modifying the CCL2/CCR2 and CCL3/CCR1 axis. *L. acidophilus* CCFM137 had different effects by down regulating the relative expression of CCL2 and CCL3 in the colon, while L. acidophilus FGSYC48L79 had negative effects by increasing harmful bac-

teria abundance and promoting chemokine signalling. *Lactobacillus plantarum* strains improved dysbiosis, increased levels of beneficial bacteria associated with SCFA production, reduced pro-inflammatory cytokines, and increased anti-inflammatory cytokines[24]. *Lactobacillus rhamnosus* strains BY-02 and *L. plantarum* BY-05 combination ("LS treatment") suppressed weight loss, improved intestinal barrier damage, altered microbial composition, increased SCFA content, and suppressed inflammatory signalling[25]. *Lactobacillus salivarius* UCC118TM reduced disease severity, upregulated anti-inflammatory markers such as upregulation of tissue IL-10 levels and increased expression of macrophage M2 markers, and accelerated recovery in DSS-induced colitis[26]. *Lactiplantibacillus plantarum* subsp. plantarum SC-5 alleviated colitis symptoms, reduced pro-inflammatory cytokines, strengthened the intestinal barrier, restored flora balance, and increased beneficial microbiota. It also attenuated the inflammatory response by inhibiting the protein expression of NF-kB and MAPK signaling pathways[27]. *Lactobacillus gasseri* G098 effectively reversed colitis-associated symptoms, prevented mortality, balanced serum cytokines, and modulated the gut microbiome[28]. These studies highlight the heterogeneity of Lactobacillus strains in mitigating colitis symptoms, improving gut microbiota composition, and regulating immune responses. The G098 treatment also significantly increased the relative abundance of gut Firmicutes while reducing the proportion of Bacteroidetes species[28].

Other probiotic strains were also assessed during the reporting period. *Clostridium butyricum* was shown to provide protection against colitis by preventing weight loss, reducing disease activity, shortening the colon, improving gut barrier function, and decreasing levels of pathogenic bacteria including *Escherichia/Shigella*. Additionally, there was an increased abundance of beneficial bacteria and butyrate-producing species detected in *C. butyricum* treated DSS-induced colitis murine model[29]. Studies confirmed that the gut microbiota and metabolites produced by *C. butyricum* played crucial roles in attenuating colitis. Furthermore, *C. butyricum*-derived extracellular vesicles (EVs) were found to protect the gut barrier function, improve gut microbiota homeostasis, and contribute to overall colitis alleviation. Another aspect of the study focused on *C. butyricum* Prazmowski (CB) specifically[30]. *C. butyricum* feeding was found to decrease disease activity scores, colon inflammation/injury score, and cell apoptosis in an experimental colitis mouse model. *C. butyricum* also elevated levels of short-chain fatty acids (SCFAs) in cecal faeces. *C. butyricum* exhibited the ability to balance inflammatory cytokines, protect tight junctions, increase the number of goblet cells, and promote MUC2 production. The study concluded that *C. butyricum* could protect the gut barrier and alleviate experimental colitis through transactivation of EGFR signalling in intestinal epithelial cells. Overall, the study shed light on the potential efficacy of *C. butyricum* as preventive strategies for IBD and their prospects as food supplements. However, in the context of the *C. butyricum* Miyairi (CBM) intervention on AOM/DSS mice, it failed to improve colitis and colitis-associated neoplasms, altered microbial composition, and unexpectedly increased proinflammatory IL-17A expression[31]. The heterogeneity between *C. butyricum* strains again highlights the need to evaluate strain specific potential of gut microbes.

A three-species consortium called BAC (bile acid consortium), consisting of *Clostridium* AP sp000509125, *Bacteroides ovatus*, and *Eubacterium limosum* was also assessed for its anti-inflammatory potential. BAC converted primary bile acids to secondary bile acids and showed protective effects against colitis[32]. Oral gavage BAC treatment reduced weight loss, increased colon length, improved intestinal barrier function, and modulated the gut microbiota. BAC also restored dysregulated bile acid metabolism, promoted the activation of the bile acid receptor TGR5, improved gut barrier integrity, and reduced inflammation. A further three-species consortium comprising *Bifidobacterium bifidum* H3-R2, *Propionibacterium freudenreichii* B1, and *Clostridium butyricum* C1-6 increased colon length, reduced weight loss, decreased splenic index, and improved disease activity index (DAI) scores and myeloperoxidase (MPO) activity in a DSS murine colitis model[33]. The strain consortium also reduced levels of proinflammatory factors (IL-8, IL-1β, and TNF-α), increased the production of the anti-inflammatory factor IL-10, and enhanced the expression of tight junction proteins (ZO-1, occludin, and claudin-1) in the colon. *B. bifidum* H3-R2 and *P. freudenreichii* B1 were shown to exert their protective effects through TLRs/RHO kinase (ROCK1) and Wnt/β-catenin pathways. Furthermore, all three strains increased the abundance of *Lactobacillus* and *Bifidobacterium* species abundance and increased the production of short-chain fatty acids (SCFAs).

Another study investigated the effects of a potential new probiotic species *Coprococcus eutactus* to reduce colitis development[34]. Administration of *C. eutactus* ameliorated colitis symptoms, including weight loss and proinflammatory cytokine reduction. It enhanced goblet cell maturation, mucin expression, and restored tight junction protein expression including claudin-1, occludin, and ZO-1. *C. eutactus* also increased levels of secretory immunoglobulin A (SIgA), which specifically coated *Enterobacteriaceae* pathogens, resulting in a restored and remodelled gut microbiota.

A further study examined the effects of heat-killed *Bifidobacterium bifidum* B1628 (HB1628) on DSS-induced colitis[35]. HB1628 administration reduced disease severity, tissue damage, and pro-inflammatory cytokine levels (IL-1 and TNF-a) while increasing anti-inflammatory cytokine levels (IL-13). It also improved gut dysbiosis through modulation of gut microbiota composition, increasing *Lactobacillus* abundance, and decreasing unfavourable taxa associated with IBD including *Porphyromonadaceae* and *Subdoligranulum*. HB1628 intervention also influenced metabolic pathways (namely, the aerobic respiration I [cytochrome c] pathway and L-tryptophan biosynthesis) and mitigated colitis severity by regulating cytokine balance and reducing inflammation in gut tissue.

One study assessed the effectiveness of *Saccharomyces cerevisiae* isolated from Tibetan kefir grains, known for its probiotic properties, in improving colitis in a murine model induced by *Fusobacterium nucleatum* infection and DSS treatment[36]. When administered to mice with experimental colitis, *S. cerevisiae* supplementation led to increased body weight and expression of anti-inflammatory cytokines (IL-4 and IL-10), while reducing disease activity and expression of proinflammatory cytokines (TNF-α, IL-6, and IL-17F). The *S. cerevisiae*-fed group also exhibited improved gut barrier function, as evidenced by increased levels of tight junction proteins and goblet cells. The mechanism behind *S. cerevisiae*'s effectiveness in ameliorating *F. nucleatum*-DSS-induced colitis involved a decrease in reactive oxygen species levels in the colon, inhibition of endoplasmic reticulum stress, and modulation of gut microbiota.

## PRECLINICAL STUDIES OF GUT MICROBIOTA CHANGES AND IBD – NON-BACTERIAL FACTORS

Sinha et al[37] investigated the role of bacteriophages in modulating gut bacterial communities and disease outcome in IBD. They performed *in vivo* cross-infection experiments in human microbiota-associated (HMA) mice by transplanting fecal virus-like particles (VLPs) isolated from UC patients and healthy controls. The VLPs isolated from healthy controls and UC patients differentially altered the composition of the gut microbiota where the UC virome was dominated by Microviridae. Furthermore, UC bacterial communities enhanced DSS colitis severity compared to bacterial communities from healthy controls.

Echinococcus granulosus sensu stricto parasite infection was also assessed for its ability to ameliorate DSS-induced colitis in a mouse model[38]. Both the average body weight and the disease activity index (DAI) score improved post E. granulosus infection. The authors then purified antigen B (AgB), which is the major source of secreted protein of E. granulosus in echinococcal cyst fluid (ECF), and re-injected it into the DSS mice, resulting in similar decreased symptoms. Focusing on the effect of AgB, they found that AgB significantly decreased iNOS expression and increased Fizz1 expression. In terms of the gut microbiota composition, five genera (*Paraprevotella, Odoribacter, Clostridium* XlVa, *Oscillibacter* and *Flavonifractor*) were significantly increased in the AgB inoculated DSS mice. Their results suggested that AgB may be a drug candidate for IBD treatment.

Deng et al[39] investigated the effects of prior colonization with two microscopic parasitic *Blastocystis* subtypes in DSS-induced colitis murine models. The microbiota composition analysis showed that prior infection with Blastocystis ST4 increased the abundance of beneficial bacteria, including *Lachnospiraceae* NK4A136 group and *Clostridia vadin* BB60 group which can produce short-chain fatty acids (SCFA); on the other hand, Blastocystis ST7 prior infection caused a reduction in beneficial bacteria *Lachnospiraceae* UCG-001 and Lactobacillus, and an increase in the sulphate-producing bacteria *Desulfovibrio*. Moreover, the increased severity of experimental colitis in the ST7-infected mice, including progressive weight loss, disease activity and shortened colon lengths, were not observed in the ST4-infected group. IL-4 and IL-10-producing CD4+ T

cells were significantly increased in the ST4-colonized mice while pro-inflammatory IL-17A and TNF-α-producing CD4+ T cells were enriched in the ST7 group. Furthermore, FMT of ST4- and ST7-altered microbiota showed similar results suggesting that the Blastocystis-altered microbiota could affect DSS-induced colitis even without *Blastocystis* present.

Ocansey et al[40] established a DSS-induced IBD model of BALB/C mice and assessed gut microbial metagenomic and metabolomic profiles to assess the regulatory effect of mesenchymal stem cell-derived exosomes (MSC-Ex). Gut bacteria community profiles in the DSS model were restored by MSC-Ex in terms of increasing the alpha diversity and levels of 'beneficial bacteria'. Functional analysis showed that MSC-Ex not only enhanced the function of key cellular activities including transcription, carbohydrate transport and metabolism, signal transduction mechanisms, cell motility, cytoskeleton, and RNA processing and modification but also decreased cancer function. Moreover, the reduced expression of bile acid receptor FXR caused by DSS-induced colitis was largely restored by MSC-Ex treatment. These factors suggest that MSC-Ex can assist in mitigating colitis in mice by modulating the gut bacterial community and led the authors to suggest that the MSC-Ex-regulated FXR could provide a potential therapeutic target for IBD.

Wang et al[41] investigated the effects of Dectin-1 and Dectin-2, which are receptors for fungal cell wall components, on DSS-induced colitis. Knockout of both Dectin-1 and Dectin-2 exhibited a strongly decreased disease severity, including lower weight loss and disease activity, however, deletion of either receptor alone did not reveal significant ameliorated effects. FMT from double knockout mice into wild-type resulted in reduced colitis severity while transplanting the wild-type microbiota into double knockout mice increased colitis severity, suggesting that the protection of double knockout against DSS-induced colitis was largely due to the gut microbiota. Combining the double knockout with antifungal drugs did not affect inflammation symptoms. 16S sequencing data analysis indicated an enrichment of the *Lachnospiraceae* family in the double knockout mice, suggesting that bacterial but not fungal gut microbiota were involved in the protection induced by Dectin-1 and Dectin-2 deficiency in intestinal inflammation.

## PRECLINICAL STUDIES OF GUT MICROBIOTA CHANGES AND IBD – NON-MICROBIAL FACTORS

Liu et al[42] utilised the DSS colitis mouse model to assess gut microbiota changes caused by sleep interruption and subsequent electroacupuncture (EA). Beta diversity analysis indicated that the EA treated group had the closest microbiota profiles to controls, with colon length and body weight significantly improved by EA compared with DSS colitis mouse without EA. According to immunohistochemical staining results, one possible explanation for the EA effect on ameliorating colitis severity was its capability of upregulating the expression of Vasoactive Intestinal Peptide (VIP) receptors hence mediating tight junction proteins reservation.

In a study undertaken by da Silva et al[43], C57BL/6 mice that were exposed to DSS and treated with MLT showed a significant increase in colitis severity compared to those treated with DSS only, suggesting that the DSS-induced inflammatory effects were potentiated by MLT. MLT regulated the immune response in the spleen and mesenteric lymph nodes, increasing the proportion of various immune cell subtypes. In terms of the impact of MLT on the faecal microbiota, MLT treatment reduced *Bacteroidetes* abundance and increased Actinobacteria and Verrucomicrobia phyla, respectively. The mice treated with antibiotics followed by MLT presented a remarkable reversion of the colitis phenotype, including a counter-regulatory immune response, reduction in tumour necrosis factor (TNF) and colon macrophages.

## CONCLUSIONS

This review details the highlights in a year of important advances in mechanistic studies of the gut microbiota and IBD. Further understanding of the modulation of gut microbiota by dietary factors including nutritional manipulations and pre/probiotic factors. The significance of non-bacterial factors of the gut microbiota continue to challenge current concepts but further studies which embrace the holistic microbiota rather than individual contributors need to be encouraged.

## Conflict of Interest

There is no conflict of interest to declare.

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## REFERENCES

1. Yang J, Germano PM, Oh S, Wang S, Wang J, Lee R, Paige H, Yang S, Henning SM, Zhong J, Jacobs JP, Li Z. Pomegranate Extract Improves Colitis in IL-10 Knockout Mice Fed a High Fat High Sucrose Diet. Mol Nutr Food Res 2022; 66: e2100730.
2. Yang W, Huang Z, Xiong H, Wang J, Zhang H, Guo F, Wang C, Sun Y. Rice Protein Peptides Alleviate Dextran Sulfate Sodium-Induced Colitis via the Keap1-Nrf2 Signaling Pathway and Regulating Gut Microbiota. J of Agric Food Chem 2022; 70: 12469-12483.
3. Asanuma N. Effect of Dietary Ceramide and Glucosylceramide on the Alleviation of Experimental Inflammatory Bowel Disease in Mice. J Oleo Sci 2022; 71: 1397-1402.
4. Liu M, Liu F, Pan Y, Xiong Y, Zeng X, Zheng L, Zhao H, Li Y, Liu D. Oxymatrine ameliorated experimental colitis via mechanisms involving inflammatory DCs, gut microbiota and TLR/NF-κB pathway. Int Immunopharmacol 2023; 115: 109612.
5. Xia X, Lin H, Luo F, Wu X, Zhu L, Chen S, Luo H, Ye F, Peng X, Zhang Y, Yang G, Lin Q. Oryzanol Ameliorates DSS-Stimulated Gut Barrier Damage via Targeting the Gut Microbiota Accompanied by the TLR4/NF-κB/NLRP3 Cascade Response In Vivo. J of Agric Food Chem 2022; 70: 15747-15762.
6. Zhang Y, Feng X, Lin H, Chen X, He P, Wang Y, Chu Q. Tieguanyin extracts ameliorated DSS-induced mouse colitis by suppressing inflammation and regulating intestinal microbiota. Food Funct 2022; 13: 13040-13051.
7. Zhao N, Yang Y, Chen C, Jing T, Hu Y, Xu H, Wang S, He Y, Liu E, Cui J. Betaine supplementation alleviates dextran sulfate sodium-induced colitis via regulating the inflammatory response, enhancing the intestinal barrier, and altering gut microbiota. Food Funct 2022; 13: 12814-12826.
8. Mo J, Ni J, Zhang M, Xu Y, Li Y, Karim N, Chen W. Mulberry Anthocyanins Ameliorate DSS-Induced Ulcerative Colitis by Improving Intestinal Barrier Function and Modulating Gut Microbiota. Antioxidants 2022; 11: 1674.
9. Feng Y, Li D, Ma C, Tian M, Hu X, Chen F. Barley Leaf Ameliorates Citrobacter rodentium-Induced Colitis through Preventive Effects. Nutrients 2022; 14: 3833.
10. Ren R, Zhao AQ, Chen L, Wu S, Hung WL, Wang B. Therapeutic effect of Lactobacillus plantarum JS19 on mice with dextran sulfate sodium induced acute and chronic ulcerative colitis. J Sci Food Agric 2022; 103: 4143-4156.
11. Liu Q, Zhang X, Li Z, Chen Y, Yin Y, Lu Z, Ouyang M, Chen L. Maternal diets have effects on intestinal mucosal flora and susceptibility to colitis of offspring mice during early life. Nutrition 2022; 99: 111672.
12. Metwaly A, Jovic J, Waldschmitt N, Khaloian S, Heimes H, Häcker D, Ahmed M, Hammoudi N, Le Bourhis L, Mayorgas A, Siebert K, Basic M, Schwerd T, Allez M, Panes J, Salas A, Bleich A, Zeissig S, Schnupf P, Cominelli F, Haller D. Diet prevents the expansion of segmented filamentous bacteria and ileo-colonic inflammation in a model of Crohn's disease. Microbiome 2023; 11: 66.

13. Lu PD, Yuan MC, Quan XP, Chen JF, Zhao YH. Preclinical studies of licorice in ulcerative colitis: A systematic review with meta-analysis and network pharmacology. J Ethnopharmacol 2022; 296: 115444.
14. Pi Y, Zhang X, Wu Y, Wang Z, Bai Y, Liu X, Han D, Zhao J, Tobin I, Zhao J, Zhang G, Wang J. Alginate Alleviates Dextran Sulfate Sodium-Induced Colitis by Promoting Bifidobacterium animalis and Intestinal Hyodeoxycholic Acid Synthesis in Mice. Microbiol Spectr 2022; 10: e0297922.
15. Chen C, Wang H, Hong T, Huang X, Xia S, Zhang Y, Chen X, Zhong Y, Nie S. Effects of tea polysaccharides in combination with polyphenols on dextran sodium sulfate-induced colitis in mice. Food Chem X 2022; 13: 100190.
16. Guo J, Ma B, Wang Z, Chen Y, Tian W, Dong Y. Royal Jelly Protected against Dextran-Sulfate-Sodium-Induced Colitis by Improving the Colonic Mucosal Barrier and Gut Microbiota. Nutrients 2022; 14 2069.
17. Dong L, Xie J, Wang Y, Jiang H, Chen K, Li D, Wang J, Liu Y, He J, Zhou J, Zhang L, Lu X, Zou X, Wang XY, Wang Q, Chen Z, Zuo D. Mannose ameliorates experimental colitis by protecting intestinal barrier integrity. Nature Commun 2022; 13: 4804.
18. Niechcial A, Schwarzfischer M, Wawrzyniak M, Atrott K, Laimbacher A, Morsy Y, Katkeviciute E, Hafliger J, Westermann P, Akdis CA, Scharl M, Spalinger MR. Spermidine ameliorates colitis via induction of anti-inflammatory macrophages and prevention of intestinal dysbiosis. J Crohns Colitis 2023; jjad058. doi: 10.1093/ecco-jcc/jjad058. Online ahead of print.
19. Fei Y, Li S, Wang Z, Ma Y, Fang J, Liu G. IRW (Ile-Arg-Trp) Alleviates DSS-Induced Intestinal Injury by Remodeling Gut Microbiota and Regulating Fecal SCFA Levels. Nutrients 2023; 15: 953.
20. Chang CC, Liu CY, Su IC, Lee YJ, Yeh HJ, Chen WC, Yu CJ, Kao WY, Liu YC, Huang CJ. Functional Plasmon-Activated Water Increases Akkermansia muciniphila Abundance in Gut Microbiota to Ameliorate Inflammatory Bowel Disease. Int J Mol Sci 2022; 23: 11422.
21. Low D, Nguyen DD, Mizoguchi E. Animal models of ulcerative colitis and their application in drug research. Drug Des Devel Ther 2013; 7: 1341-1357.
22. Cheng D, Xie MZ. A review of a potential and promising probiotic candidate-Akkermansia muciniphila. J Appl Microbiol 2021; 130: 1813-1822.
23. Liu Y, Yang M, Tang L, Wang F, Huang S, Liu S, Lei Y, Wang S, Xie Z, Wang W, Zhao X, Tang B, Yang S. TLR4 regulates RORγt(+) regulatory T-cell responses and susceptibility to colon inflammation through interaction with Akkermansia muciniphila. Microbiome 2022; 10: 98.
24. Khan I, Wei J, Li A, Liu Z, Yang P, Jing Y, Chen X, Zhao T, Bai Y, Zha L, Li C, Ullah N, Che T, Zhang C. Lactobacillus plantarum strains attenuated DSS-induced colitis in mice by modulating the gut microbiota and immune response. Int Microbiol 2022; 25: 587-603.
25. Liu Y, Zhang H, Xie A, Sun J, Yang H, Li J, Li Y, Chen F, Mei Y, Liang Y. Lactobacillus rhamnosus and L. plantarum Combination Treatment Ameliorated Colitis Symptoms in a Mouse Model by Altering Intestinal Microbial Composition and Suppressing Inflammatory Response. Mol Nutr Food Res 2023: e2200340.
26. Iyer N, Williams MA, O'Callaghan AA, Dempsey E, Cabrera-Rubio R, Raverdeau M, Crispie F, Cotter PD, Corr SC. Lactobacillus salivarius UCC118™ Dampens Inflammation and Promotes Microbiota Recovery to Provide Therapeutic Benefit in a DSS-Induced Colitis Model. Microorganisms 2022; 10.
27. Shi R, Yu F, Hu X, Liu Y, Jin Y, Ren H, Lu S, Guo J, Chang J, Li Y, Liu Z, Wang X, Hu P. Protective Effect of Lactiplantibacillus plantarum subsp. plantarum SC-5 on Dextran Sulfate Sodium-Induced Colitis in Mice. Foods 2023; 12: 897.
28. Zhang WQ, Quan KY, Feng CJ, Zhang T, He QW, Kwok LY, Chen YF. The Lactobacillus gasseri G098 Strain Mitigates Symptoms of DSS-Induced Inflammatory Bowel Disease in Mice. Nutrients 2022; 14: 3745.
29. Ma L, Shen Q, Lyu W, Lv L, Wang W, Yu M, Yang H, Tao S, Xiao Y. Clostridium butyricum and Its Derived Extracellular Vesicles Modulate Gut Homeostasis and Ameliorate Acute Experimental Colitis. Microbiol Spectr 2022; 10: e0136822.
30. Wu J, Zhou B, Pang X, Song X, Gu Y, Xie R, Liu T, Xu X, Wang B, Cao H. Clostridium butyricum, a butyrate-producing potential probiotic, alleviates experimental colitis through epidermal growth factor receptor activation. Food Funct 2022; 13: 7046-7061.
31. Hu ML, Lian WS, Wang FS, Yang CH, Huang WT, Yang JW, Chen IY, Yang MY. Presume Why Probiotics May Not Provide Protection in Inflammatory Bowel Disease through an Azoxymethane and Dextran Sodium Sulfate Murine Model. Int J Mol Sci 2022; 23: 9689.
32. Zhou C, Wang Y, Li C, Xie Z, Dai L. Amelioration of Colitis by a Gut Bacterial Consortium Producing Anti-Inflammatory Secondary Bile Acids. Microbiol Spectr 2023; 11: e0333022.
33. Yang S, Shang J, Liu L, Tang Z, Meng X. Strains producing different short-chain fatty acids alleviate DSS-induced ulcerative colitis by regulating intestinal microecology. Food Funct 2022; 13: 12156-12169.
34. Yang R, Shan S, Shi J, Li H, An N, Li S, Cui K, Guo H, Li Z. Coprococcus eutactus, a Potent Probiotic, Alleviates Colitis via Acetate-Mediated IgA Response and Microbiota Restoration. J of Agric Food Chem 2023. doi: 10.1021/acs.jafc.2c06697. Online ahead of print.
35. Feng C, Zhang W, Zhang T, He Q, Kwok LY, Tan Y, Zhang H. Heat-Killed Bifidobacterium bifidum B1628 May Alleviate Dextran Sulfate Sodium-Induced Colitis in Mice, and the Anti-Inflammatory Effect Is Associated with Gut Microbiota Modulation. Nutrients 2022; 14: 5233.
36. Zeng X, Li X, Yue Y, Wang X, Chen H, Gu Y, Jia H, He Y, Yuan Y, Yue T. Ameliorative Effect of Saccharomyces cerevisiae JKSP39 on Fusobacterium nucleatum and Dextran Sulfate Sodium-Induced Colitis Mouse Model. J Agric Food Chem 2022; 70: 14179-14192.
37. Sinha A, Li Y, Mirzaei MK, Shamash M, Samadfam R, King IL, Maurice CF. Transplantation of bacteriophages from ulcerative colitis patients shifts the gut bacteriome and exacerbates the severity of DSS colitis. Microbiome 2022; 10: 105.

38. Bao J, Qi W, Sun C, Tian M, Jiao H, Guo G, Guo B, Ren Y, Zheng H, Wang Y, Yan M, Zhang Z, McManus DP, Li J, Zhang W. Echinococcus granulosus sensu stricto and antigen B may decrease inflammatory bowel disease through regulation of M1/2 polarization. Parasit Vectors 2022; 15: 391.
39. Deng L, Wojciech L, Png CW, Kioh DYQ, Gu Y, Aung TT, Malleret B, Chan ECY, Peng G, Zhang Y, Gascoigne NRJ, Tan KSW. Colonization with two different Blastocystis subtypes in DSS-induced colitis mice is associated with strikingly different microbiome and pathological features. Theranostics 2023; 13: 1165-1179.
40. Ocansey DKW, Zhang Z, Xu X, Liu L, Amoah S, Chen X, Wang B, Zhang X, Mao F. Mesenchymal stem cell-derived exosome mitigates colitis via the modulation of the gut metagenomics-metabolomics-farnesoid X receptor axis. Biomater Sci 2022; 10: 4822-4836.
41. Wang Y, Spatz M, Da Costa G, Michaudel C, Lapiere A, Danne C, Agus A, Michel ML, Netea MG, Langella P, Sokol H, Richard ML. Deletion of both Dectin-1 and Dectin-2 affects the bacterial but not fungal gut microbiota and susceptibility to colitis in mice. Microbiome 2022; 10: 91.
42. Liu GH, Zhuo XC, Huang YH, Liu HM, Wu RC, Kuo CJ, Chen NH, Chuang LP, Lin SW, Chen YL, Yang HY, Lee TY. Alterations in Gut Microbiota and Upregulations of VPAC2 and Intestinal Tight Junctions Correlate with Anti-Inflammatory Effects of Electroacupuncture in Colitis Mice with Sleep Fragmentation. Biology 2022; 11: 962.
43. da Silva JL, Barbosa LV, Pinzan CF, Nardini V, Brigo IS, Sebastião CA, Elias-Oliveira J, Brazão V, Júnior JCDP, Carlos D, Cardoso CRB. The Microbiota-Dependent Worsening Effects of Melatonin on Gut Inflammation. Microorganisms 2023; 11: 460.