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Immunological mechanisms underpinning faecal microbiota transplantation for the treatment of inflammatory bowel disease

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Summary

Inflammatory bowel disease (IBD) is a chronic gastrointestinal disease that results from a dysregulated immune response against specific environmental triggers in a genetically predisposed individual. Increasing evidence has indicated a causal role for changes in gut microbiota (dysbiosis) contributing to this immune-mediated intestinal inflammation. These mechanisms involve dysregulation of multiple facets of the host immune pathways that are potentially reversible. Faecal microbiota transplantation (FMT) is the transfer of processed stool from a healthy donor into an individual with an illness. FMT has shown promising results in both animal model experiments and clinical studies in IBD in the resolution of intestinal inflammation. The underlying mechanisms, however, are unclear. Insights from these studies have shown interactions between modulation of dysbiosis via changes in abundances of specific members of the gut microbial community and changes in host immunological pathways. Unravelling these causal relationships has promising potential for a translational therapy role to develop targeted microbial therapies and understand the mechanisms that underpin IBD aetiopathogenesis. In this review, we discuss current evidence for the contribution of gut microbiota in the disruption of intestinal immune homeostasis and immunoregulatory mechanisms that are associated with the resolution of inflammation through FMT in IBD.

Keywords: faecal microbiota transplantation, immunology, inflammatory bowel disease

Introduction

Inflammatory bowel disease (IBD) is a chronic, immune-mediated gastrointestinal condition that affects more than 1 million of the UK population [1]. It broadly comprises two diseases: ulcerative colitis (UC) and Crohn's disease (CD). Patients typically present with a constellation of symptoms that include chronic diarrhoea, rectal bleeding and abdominal pain as a consequence of chronic inflammation [2]. Traditionally, IBD was considered to be an autoimmune disease. It is now increasingly accepted that it is the result of a dysregulated immune response against specific environmental triggers in genetically predisposed individuals [3].

This critical significance of environmental influences is further supported by growing recognition of the fundamental role of the gut microbiota in the development and progression of inflammation in IBD [4]. The gut microbiota exists as a complex multi-cellular community that, in health, lives synergistically with its host [5]. This community, consisting of bacteria, viruses, fungi, archaea and other protists, plays a crucial role in influencing host physiology in health and disease. Shifts in the composition and function of gut microbiota (known as dysbiosis) have been associated with a multitude of immune-mediated chronic diseases of the gastrointestinal tract, including IBD and primary sclerosing cholangitis [6]. From data accruing

in studies exploring multiple aspects of host immune interactions with gut microbiota and their manipulation, we are progressively uncovering key mechanisms of causality and discovering novel treatment targets for IBD [7]. In this review, we discuss evidence for the causal role of gut microbiota in driving proinflammatory pathways in IBD and the immunological mechanisms that restore homeostatic balance as a consequence of its manipulation by faecal microbiota transplantation (FMT).

Host immune microbiota relationships govern IBD

The gut faces the exceptional challenge of maintaining intestinal immune tolerance and host mutualism to the vast and diverse commensal microbiota while mounting an appropriate defence to pathogens. Host immune cells, in conjunction with the intestinal barrier, manage this through a variety of immunological mechanisms that, in addition to the cellular and humoral immune responses, include mucus secretion, immunoglobulin (Ig)A and antimicrobial peptides. This immune homeostasis, in turn, facilitates the maintenance of a relatively stable gut microbial community while limiting the colonization of pathogenic organisms. Dysregulation of many facets of the mucosal immune homeostasis is the cardinal feature that drives disease in IBD. Genomewide association studies in IBD demonstrate variants among candidate genes involved in multiple immune pathways, including antigen-sensing, immune cell trafficking and pathogen handling [8,9]. With the alarming rate of increase in the global incidence and prevalence of IBD, it appears more likely that environmentally derived immune triggers are driving the development of IBD, rather than a seeming unlikely rapid increase in the pool of these gene variants. Regions in Asia, which are currently viewing the highest incidence of IBD, are genetically distinct from western countries, which were deemed traditionally to be high-risk areas [10].

Epidemiological studies have identified a number of environmental influences associated with the development of IBD, ranging from events at birth and exposure to antibiotics in early life to a western diet [11–13]. Although no direct causality can be associated with these factors in the disruption of mucosal immune homeostasis it is clear that, individually, these have a major effect on the development and maintenance of gut microbiota. Studies have consistently shown that patients with IBD have significant dysbiosis compared to those without disease. Expansion of potential pathogens as well as global reduction in the symbiotic species and compositional diversity have been consistently described. Members of the phylum Firmicutes, specifically *Faecalibacterium prausnitzii*, have been shown to be reduced in both stool and mucosal biopsies, while the phylum Proteobacteria comprising species such as *Escherichia coli* and other members of the *Enterobacteriaceae* family are found to be increased in patients with IBD when compared to healthy individuals [4,14–17]. These alterations appear to be represented by a reduced abundance of butyrate-producing species that include *Blautia faecis*, *Roseburia inulinivorans*, *Ruminococcus torques* and *F. prausnitzii*, and an increase in sulphate-reducing bacteria such as *Desulfovibrio* and other species with proinflammatory properties, such as adhesion-invasive *E. coli* (AIEC) in IBD [18–20]. Collectively and conistently these findings strongly indicate that the alteration of gut microbiota is associated with the pathogenesis of IBD. Whether this is a primary or secondary event and if the mucosal immune response is appropriate or exaggerated are two of the fundamental questions that remain to be answered [21]. While a single causative agent has remained elusive, the origin of IBD is likely to be a consequence of an aberrant host immunological response (influenced partly by genetic predisposition) of an environmentally dictated shift in the gut microbiota.

Evidence of the causal role of dysbiosis for proinflammatory innate and adaptive immune responses in IBD

There is considerable evidence demonstrating complex dynamic and bi-directional mechanistic relationships between gut microbiota and immune-mediated inflammatory responses which contribute to the pathogenesis of IBD.

Innate immunity

The gut mucosal innate immune response is ostensibly directed towards one or many foreign antigens and pathogens [22,23]. Microbial sensing occurs through Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NOD)-like receptors (NLRs) that recognize pathogen-associated molecular patterns (PAMPs) such as flagellin, lipopolysaccharide (LPS) and muramyl dipeptide (MDP). Gene polymorphisms of these receptors, together with increased expression in innate immune cells, have been described in patients with IBD [24–26]. Specific TLRs are associated with induction of either inflammatory or anti-inflammatory responses. Mucosal macrophages and dendritic cells demonstrate up-regulation of TLR-2 and TLR-4 as well as CD40 and chemokine receptor C-C chemokine receptor type 7 (CCR7) in patients with UC and CD compared to healthy controls [27]. This consequently promotes inflammation through increased production of proinflammatory cytokines such as interleukin (IL)-1, IL-6, tumour-necrosis factor (TNF)-α, IL-18 and members of the IL-12 family. Colonization of germ-free (GF) mice with a complex gut microbiota augments the expression of TLR-2 and is partly reversed by broad-spectrum antibiotics [28]. This effect appears to be bi-directional, as

expression of a flagellin-sensing transmembrane receptor, TLR-5, regulates the composition and localization of the intestinal microbiota [29]. TLRs also have the ability to promote intraepithelial cells (IECs) and Paneth cells to produce anti-microbial proteins such as regenerating gene family protein III (RegIII)β/γ that can kill Gram-positive bacteria following microbial–epithelial contact [30,31]. Patients with IBD have increased expression of Reg proteins, suggestive of compensatory defensive mechanisms against enteric pathogens [32].

A further component of the innate immune response that plays an important role in maintenance of gut mucosal homeostasis and tissue repair includes the family of innate lymphoid cells (ILC). These innate immune cells belong to the lymphoid lineage and have certain functional similarities with the adaptive CD4 T helper (Th) cell populations, but do not possess an antigen-specific T or B cell receptor. Patients with IBD have altered abundances and functionality of the different ILC subsets in the gut compared to healthy individuals [33,34]. Many key genes related to ILC3 (innate counterpart to Th17 cells) biology were identified as IBD risk loci in genomewide association studies (GWAS) and are involved in the IL-23/IL-17 pathway [35]. ILC3 subpopulations have been shown to shape microbial communities by either modifying epithelial function or the functional properties of other cells that influence microbiota composition. Certain tryptophan-based microbial metabolites were shown to directly control functionality of ILC3 through activation of ligand-dependent transcription factors, in particular aryl hydrocarbon receptor (AhR) [36]. Mice with a deletion of MHC-II in ILC3 developed spontaneous colitis in the presence of commensal bacteria [37].

Adaptive immunity

In addition to the initial contact between the environment and immune system through the innate immune pathway, the adaptive immune system also plays a crucial role in the progression of chronic inflammation in IBD [38,39]. $CD4^+$ T cell subsets play central roles in the formation of cytokine networks in IBD pathogenesis. Seminal work in the early 1990s demonstrated that adoptive transfer of CD4 T cells failed to induce colitis in recombination activating gene $(RAG)^{-/-}$ mice (deficient in B and T cells) with reduced bacterial load or raised under GF conditions [40,41]. Furthermore, CD4 T cells isolated from mice that develop spontaneous colitis were strongly reactive to MHC-II antigens from gut commensals but not to epithelial or food antigens [42]. Adoptive transfer of bacterial antigen-activated $CD4^+$ T cells from these mice into severe combined immunodeficient (SCID) mice was able to induce colitis when activated by gut microbial antigens. These findings suggested that intestinal inflammation is driven by resident gut bacteria.

The plasticity and adaptability of Th cells based on host environmental factors makes them highly relevant in the development and pathogenesis of IBD. Historically, T cell subsets were described as either Th1 cells that secrete interferon (IFN)-γ, essential for eradication of intracellular pathogens, or Th2 cells that secrete IL-4, -5 and -13 and play an essential role in the response against parasites and the fibrosis process. Consequently, based on cytokine production, CD was traditionally considered to be primarily a Th1 condition and UC was characterized as a Th2-mediated disease [3]. The discovery of IL-17-producing Th17 cells and immunomodulating regulatory T cells (T_{reg}) led to the refinement of this paradigm. Among these subsets of $CD4^+$ T cells, Th17 cells have been shown to have critical roles in mucosal defence and in the pathogenesis of autoimmune diseases [43,44]. These cells, tohether with expression of IL-17, were shown to be highly enriched in the intestinal lamina propria of patients with IBD [45]. Th17 cells are not found in the intestines of GF-reared mice, suggesting that gut bacteria are possibly responsible for generation of this immune subset [46]. Segmented filamentous bacteria (SFB) or *Candidatus savagella* have been shown to induce retinoic acid-related orphan receptor gamma t $(RORγt)^+$ Th17 subsets in mice, and this is likely to occur through a mechanism independent of TLR, NOD and adenosine 5′-triphosphate (ATP) signalling [46–48]. SFB is also a potent stimulus of the mouse intestinal IgA response and induces the recruitment of intraepithelial lymphocytes (IEL) [49–51]. Although SFBs are present in rodents, chicken and fish, direct evidence of human SFB is lacking. Apart from data fron a handful of quantitative polymerase chain reaction (qPCR), 16s rRNA and histology studies, bioinformatics searches through the Human Microbiome Project (HMP) database and other human metagenomic databases for human SFB genes have been negative [52]. More recently, other human symbiont bacterial species such as *Bifidobacterium adolescentis* have been shown to drive Th17 differentiation in mice [53,54].

A breakdown in the homeostatic adaptive immune inflammatory and regulatory mechanisms appears to play a fundamental role in the development of IBD. The development of tolerance to gut commensals is fundamental to the induction and maintenance of a host–microbial mutualistic T cell response, thereby limiting microbe-triggered gut inflammation [55]. $IL\text{-}10^{-/-}$, $IL\text{-}2^{-/-}$ and $IL\text{-}2R^{-/-}$ mice that have dysfunctional or reduced T_{reg} frequencies develop colitis and are commonly used models of IBD [56]. Although GF $IL\text{-}10^{-/-}$ mice do not develop colitis, introduction of specific strains of bacteria such as *Lactobacillus plantarum* 299V into specific pathogen-free $IL\text{-}10^{-/-}$ mice had a protective effect against the development of colitis, whereas strains of *Enterococcus faecalis*

induced colitis [57–59]. Interestingly, and perhaps paradoxically, multiple reports have demonstrated that T_{regs} represent a greater fraction of the lamina propria mononuclear cells (LPMC) in the intestines of IBD patients compared to healthy controls [60,61]. T_{regs} are even more common in actively inflamed than non-inflamed mucosa. Moreover, IBD patients with high proinflammatory cytokines have been shown to demonstrate an increased prevalence of dual lineage with IL-17-secreting FoxP3-expressing $CD4^+$ T cell subsets in the circulation and tissue [62,63]. Whether these T_{regs} have inherent functional deficits in immunoregulatory activity or are T_{regs} demonstrating Th17 plasticity is yet to be determined [64,65]. Mechanisms by which microbial stimuli result in T_{reg}–Th17 conversion via TLR-2 signalling have been highlighted [66]. Certainly, the importance of microbiota in influencing T_{reg}-mediated intestinal homeostasis has been described in several key studies. Transfer of specific *Clostridia* strains derived from human stool into GF mice induced a threefold increase in T_{regs} [67]. This T_{reg} increase was significantly higher with the transfer of 30 strains compared to a single strain highlighting the role of microbial diversity in host immune responses in IBD [68]. These strains were able to induce important anti-inflammatory molecules, including IL-10, transforming growth factor (TGF)-β1, cytotoxic T lymphocyte antigen 4 (CTLA-4) and inducible T cell co-stimulator (ICOS), in addition to the production of immunoregulatory short chain fatty acids (SCFA). Species from these SCFA-producing *Clostridiales* species have been shown to be reduced in the gut microbiota in patients with IBD, supporting the gut microbiota-mediated T_{reg} functionality hypothesis [69]. A recent pivotal study demonstrated that transfer of microbiota from IBD patients into GF mice significantly increased numbers of intestinal Th17 cells and decreased numbers of $ROR\gamma t^+$ T_{reg} cells compared to microbiota from healthy individuals [70]. Furthermore, colonization with microbiota derived from IBD patients exacerbated colitis in a T cell transfer $Rag1^{-/-}$ mouse model, and the disease status correlated with an increase in microbiota-induced proportions of Th17 and $ROR\gamma t^+$ T_{reg} cells compared to mice colonized with healthy donor microbiotas. These findings collectively highlight the role of microbiota in determining intestinal Th17 and $ROR\gamma t^+$ T_{reg} cell compartments as an important mechanism of pathogenesis in IBD.

A variety of innate and adaptive immune mechanisms are known to influence immunoglobulin responses to the intestinal microbiota [71,72]. Increased infiltration of intestinal mucosal plasma cells and mucosal immunoglobulin levels provide further compelling evidence for gut mucosal–microbial interaction in the pathogenesis of IBD. High levels of mucosal IgG directed against commensal bacterial antigens have been described in the gut in IBD and these appear to be principally directed against the bacterial cytoplasmic rather than the membrane proteins [73,74]. Patients with UC appear to have higher serum IgG responses to species including *Peptostreptococcus anaerobius* strains, *E. faecalis*, *Streptococcus bovis* and specific bacteria from the *Clostridia* class, and these antibodies greatly enhanced the respiratory burst in polymorphonuclear neutrophils in response to bacterial species [75]. A key study that performed 16S rRNA sequencing of IgA-coated intestinal microbiota (IgA-seq) isolated from stool in IBD patients discovered that 35 species were uniquely highly coated in patients with IBD, and this was often independent of differences in abundances [76]. These IgA-inducing members of the intestinal microbiota cultured from IBD patients exacerbated dextran sulphate sodium (DSS) colitis in GF mice, thereby highlighting its causal role in susceptibility to colitis.

Exploring gut microbiota manipulation for treatment of IBD

As no single causative trigger has been yet identified, similar to most immune-mediated diseases, treatment of IBD is primarily directed towards suppression of host immunological consequences. Current treatments for IBD are focused on counteracting multiple facets of these immune pathways. Drugs blocking pleiotropic proinflammatory pathways, such as steroids, immunomodulatory drugs, anti-TNF agents, the Th1/Th17 axis via IL-12/23 blockage and leucocyte trafficking via anti-integrins and signalling molecules, are the mainstay of medical management of IBD [77]. These agents are fairly effective, with steroid-free clinical remission rates ranging from 57% for combination therapy with infliximab (anti-TNF) and azathioprine (thiopurine) to 18% for tofactinib [targets Janus kinase/signal transducers and activators of transcription (JAK/STAT) signalling pathway] [78]. With increasing evidence of a dysregulated intestinal microbiome being a probable immunological trigger for inflammation in IBD, exploring therapeutic manipulation of the gut microbiome is an attractive strategy to identify new treatment options, and help understand the underlying cause of IBD.

Microbial manipulation in IBD has been evaluated through several approaches. A fairly untargeted approach using antibiotics has only proved successful in treating inflammation that occurs in the distal small bowel that has been refashioned into a pouch following removal of the large bowel for colitis – known as pouchitis [79]. There is limited evidence for the role of antibiotics in mild to moderate CD, with questionable long-term benefits [80]. Certainly, antibiotic-related adverse events, unknown long-term consequences associated with alterations in the microbiome and the emergence of anti-microbial resistant genes makes this strategy of limited attractiveness. Targeted modulation of gut microbiota has been explored with the use of probiotics. These are live microorganisms that

mediate their effects in treating IBD through potentially up-regulating anti-inflammatory immune pathways. Results in clinical trials in IBD have been mixed, with meta-analyses only showing benefit in modest prevention of relapse only for UC when used in conjunction with 5-aminosalicylates and moderate-quality evidence highlighting its role in the prevention of pouchitis [81,82].

One of the probable reasons for the lack of success with antibiotics and probiotics is that these approaches tend to assume that microbial triggers for IBD follow Koch's postulates, and attempt an untargeted change of the underlying dysbiosis. However, if we assume that the underlying mechanism stimulating IBD is a shift in the gut microbial community towards an ecosystem that induces and maintains host proinflammatory pathways, then re-establishing this community towards a 'healthy' population of microbes should, in effect, restore immunological homeostasis. FMT is the transfer of a processed stool obtained from a healthy donor into a patient with the aim of correcting the underlying dysbiosis by attempting to restore the intestinal microbial community [83,84]. FMT has proved to be highly successful in treating recurrent and antibiotic refractory *Clostridiodes difficile* (*C. difficile*) infection (CDI), with cure rates approaching 90% [85]. Unlike IBD, the mechanisms of disease in CDI are better understood where toxins and other putative virulence factors produced by *C. difficile* are responsible for the pathogenicity [86].

The obvious success of FMT in CDI has resulted in the exploration of its use in treatment of IBD. Four randomized controlled trials and a multitude of case reports have been completed to date, primarily evaluating the efficacy of FMT in UC. A meta-analysis of the four randomized controlled trials (RCTs) has demonstrated that clinical remission was achieved in 39 of 140 (28%) patients in the donor FMT groups compared with 13 of 137 (9%) patients in the placebo groups ($P < 0{\cdot}01$) [87]. There was marked variability in the designs of each of these four clinical trials, ranging from differences in the route of administration of FMT [upper gastrointestinal (GI) *versus* lower GI, fresh *versus* frozen], the total number of FMTs administered (two to 40 infusions), FMT preparation (anaerobic *versus* aerobic) and differences in definition of primary outcomes. Consequently, the clinical remission rates varied widely, from 24 to 50% in the FMT arm.

Immunological mechanistic insights for FMT from clinical studies in IBD

A considerable amount of mechanistic work that was incorporated into both the clinical trials and cohort studies primarily focused on changes in the recipient gut microbial and metabolomic profiles and its relationship with clinical outcomes, with little consideration of the host biological response. A general theme of an increased α-diversity or richness in the microbiome and a shift of recipient microbial profiles towards those of donors were observed, with some studies suggesting that colonization by specific donor-derived taxa was associated with clinical benefit [88]. However, immunological consequences of FMT in IBD have been very poorly described. Immunophenotyping of LPMC and peripheral blood mononuclear cells (PBMC) in patients recruited in the RCT conducted by Costello and colleagues was described in supplementary data [89]. They failed to find any significant change in proportions of γδ T cells, natural killer (NK) cells and T cells (including subsets: memory, CD4, CD8 or T_{regs}) in LPMCs as a result of FMT. Interestingly, however, on analysis of PBMCs they found a slight increase in gut-homing CD4 T cells (defined as $CD4^+CD45RO^+\beta7^+$) cells following FMT, which was only just significant ($P = 0{\cdot}05$) when adjusted for clinical disease activity scores. The gut-homing T_{reg} subset in PBMCs, however, did not change post-FMT. A detailed methodology used for cell isolation and immunophenotyping (including representative gating strategy) was not described. It also was not clear whether any shifts in immune subsets were seen in those who responded to FMT compared to those who did not. Analysis of immunological changes was not explored in any of the other three published randomized controlled trials.

There are more than 40 case–series exploring the efficacy of FMT in IBD, only a handful of which have reported immunology outcomes. A pilot study of 19 patients with active CD demonstrated an increase in the proportion of colonic mucosal T_{regs} (defined as $CD4^+CD25^+CD127^{lo}$) 12 weeks after a single colonic infusion of FMT [90]. The change was not different in responders compared to non-responders (mean 5 *versus* 5·3%, respectively) or in a Th17-like cell population ($CD4^+CD39^+CD161^+$). A study of 19 patients with moderate to severe UC failed to show a change in a large panel of serum cytokines, including IL-10 and IL-17 following a single upper GI infusion of FMT regardless of clinical response [91]. An open-label pilot of a single FMT delivery by colonoscopy in 20 patients with active UC revealed a decrease in colonic mucosal Th1 and T_{reg} cells, with no difference in the Th17 cell population post-FMT [92]. Seven patients in this study achieved clinical remission; however, a subgroup analysis of mucosal immunophenotype in these responders was not presented. A case–series that failed to show a beneficial clinical response in eight patients with chronic pouchitis after a single intragastric dose of FMT also, perhaps not surprisingly, failed to demonstrate any change in lamina propria dendritic cell phenotype and cytokine profiles [93]. A seminal study that incorporated an open-label pilot of FMT explored the role of bacteriophages in altering mucosal immune responses. In this study, 20 patients with active UC treated with a single colonoscopic infusion of FMT

showed a positive correlation between $CD4^+$ T cell production of IFN-γ from rectal mucosal biopsies and the relative abundance of total gut viral reads, specifically the bacteriophage *Caudiovirales* [94]. They found that this bacteriophage was significantly enriched in patients who failed to respond to FMT. Mechanistically, they demonstrated that introduction of *Lactobacillus*, *Escherichia* and *Bacteroides* bacteriophages into GF mice led to immune cell expansion and stimulated IFN-γ via the nucleotide-sensing receptor TLR-9 in the gut.

Immune checkpoint inhibitor (ICI) targeting CTLA-4, programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1) has been shown to improve survival among numerous cancer types by increasing T cell activation and driving an effective anti-tumour immune response. A common adverse reaction associated with ICI is colitis, which can be quite severe and resemble that seen in IBD. A case report of two patients with steroid and biologic refractory ICI-associated colitis demonstrated a successful response with FMT [95]. These patients had an increased abundance of pathogenic Gammaproteobacteria and a notable absence of potentially protective Bacteroidia. This immunological response after FMT was associated with a substantial reduction in the colonic mucosal $CD8^+$ T cell density and increase in $FoxP3^+$ CD4 cells. There was a concomitant expansion in the population of *Clostridia* and *Blautia*. There was also an increase in *Bifidobacterium* species following FMT, and this was recently reported to abrogate ICI-related toxicity in a murine model [96]. In this study, mice with DSS-induced colitis and anti-CTLA-4 blockade treatment with *Bifidobacterium* ameliorated colitis. This protective effect was abrogated in T_{reg}-depleted mice. Collectively, these findings indicate an emerging role of FMT and specific agents in the gut microbiota in mitigating inflammation via induction or modulation of T_{reg} function.

It is clear that immunological outcomes have been poorly explored in clinical FMT studies in IBD, with studies focusing primarily on gut microbial analysis. Nevertheless, these analyses have provided valuable indirect insights into microbial interactions that potentially modulate immune-mediated inflammatory responses. Gut microbiota profiles in FMT responders consistently show a significant shift towards butyrate-producing species of bacteria that are known to induce T_{regs} and promote IL-10 production [67,68]. Rossen and colleagues reported that at 12 weeks of follow-up, the microbiota of responders in the FMT group was similar to that of their healthy donors and remission was associated with proportions of *Clostridium* clusters IV and XIVa [97]. The FOCUS trial conducted by Paramsothy and colleagues reported that increased abundances of species belonging to *Clostridium* clusters XIVa and XVIII, such as *R. intestinalis*, were associated with positive outcomes following FMT [98,99]. Furthermore, members of *Clostridium* clusters IV and XIVa in the *Ruminococcaceae* and *Lachnospiraceae* families were significantly enriched in stool of a donor (donor B) who was associated with the highest rate of response following FMT in recipients in the study conducted by Moayyedi and colleagues [100]. In the analysis of mechanistic outcomes from this study, a trend was seen for responders having microbiota that was more similar to donor B than non-responders.

Immunological mechanistic insights for FMT in IBD using animal studies

Immune responses to FMT have been explored in a few studies using IBD and non-IBD mouse models. Functional effects of therapeutic FMT administration during experimental colitis on innate and adaptive immune responses in the intestinal mucosa were explored in a pivotal study [101]. Mucus and faeces derived from normobiotic mice were gavaged into $CXCR6^{EGFP+}$ reporter mice (for fluorescent T cell tracking) with DSS-induced colitis, resulting in reduction of intestinal inflammation and histological inflammation scores compared to control DSS-colitis mice. FMT-treated DSS-colitis mice demonstrated higher amounts of colonic IL-10 as well as increased frequencies of IL-10-producing $CD4^+$ T cells and invariant NK T cells (iNK T) in comparison to control DSS-treated mice. FMT treatment was also associated with a reduction of macrophages and neutrophils, together with a non-significant increase in the frequency of $FoxP3^+T_{reg}$ cells. Intriguingly, this increase in IL-10 secretion by T cells normalized upon resolution of inflammation. Pharmacological blockade of IL-10 receptor hampered the protective effects of FMT, suggesting a direct contribution of the microflora in IL-10-mediated control of inflammation. Furthermore, there was a decrease in innate lymphocytes ILC2 and ILC3, macrophages and neutrophils in the lamina propria in FMT-treated DSS-colitis mice. FMT induced a significant reduction in the number and level of expression of colonic MHC-II-expressing antigen-presenting cells (APC) (including dendritic cells and macrophages). LPMC exposed to FMT-derived microbiota showed reduced levels of proinflammatory cytokines such as TNF-α, IL-1β and IFN-γ. Camp and S100A8, two anti-microbial peptides playing anti-inflammatory roles during acute intestinal inflammation, were up-regulated upon FMT administration. Gut microbiota analysis of FMT-treated mice showed significant increases of commensals, including species belonging to *Lactobacillaceae* and *Streptococcus*, together with the SCFA-producing taxa *Erysipelotrichaceae* and *Ruminococcaceae*. This instrumental work demonstrated the beneficial anti-inflammatory effect mediated by FMT in modifying immune cell frequencies, reduction of proinflammatory colonic IFN-γ and IL-1β, increase in specific anti-microbial

peptides and mucins and a decrease of MHC-II antigen presentation by APC. Crucially, FMT induced a shift towards a tolerogenic IL-10-secreting cytokine profile that ameliorated intestinal inflammation.

In a separate study, FMT was shown to up-regulate the expression of aryl hydrocarbon receptor (AhR), IL-10 and TGF-β in colon tissues in mice with DSS-induced colitis and was associated with improvement in the severity of colon mucosa injury and histological parameters [102]. This correlated with gut microbial recovery of *Lactobacillus* and *Bifidobacterium* species and an increase in tryptophan levels which, as demonstrated by this study, results in differentiation of immune cells in order to promote or regulate the release of anti-inflammatory factors [103–105].

Administration of FMT to mice following perturbation of gut microbiota with 8 weeks of broad-spectrum antibiotics led to the re-establishment of small intestinal $CD4^+$, $FoxP3^+$ $CD8^+$ and $B220^+$, as well as of colonic $CD4^+$ and $FoxP3^+$ cell numbers as early as 7 days post-FMT [106]. Antibiotic treatment resulted in reduced cytokine production (IFN-γ, IL-17, IL-22 and IL-10) by $CD4^+$ T cells. These effects were, however, completely restored following FMT. Seven days post-FMT, a strong IL-10 response was observed in the colon, which at 28 days reached levels similar to antibiotic untreated control mice. A follow-up study showed that introduction of gut commensals *E. coli* and *L. johnsonii* increased the frequencies of T_{regs}, activated dendritic cells and intestinal memory/effector T cell populations 28 days after antibiotic-induced microbiota depletion [107]. This effect was inferior to that seen with FMT, and only *L. johnsonii* was able to maintain colonic IL-10 production. In another antibiotic-induced dysbiosis model of BALB/c mice, intragastric FMT resulted in earlier reductions of α-defensins 5 and 6 together with an increase in β-defensin 2 and concentration of secretory IgA in comparison to those that recovered spontaneously [108].

Administration of FMT following ileocolic resection in an $IL\text{-}10^{-/-}$ murine model prevented ileal inflammation but worsened colitis, and was associated with increases in colonic mucosal TNF-α, IFN-γ and IL-2 levels compared to non-operative controls. These paradoxical findings are interesting, and support the IL-10-dependent regulatory immune-mediated mechanisms of FMT that appear to be abrogated in an IL-10 knock-out model of IBD [109]. A study with anti-retroviral-treated, chronically simian immunodeficiency virus (SIV)-infected rhesus macaques that received antibiotics showed significant increases in the number of peripheral Th17 and Th22 cells following administration of FMT. Reduced $CD4^+$ T cell activation [based on expression of human leucocyte antigen D-related (HLA-DR)] in gastrointestinal tissues was also observed, and this correlated negatively with an abundance of butyrate producer *Roseburia* [110].

Mechanisms of FMT-induced immune regulation of colonic inflammation

The evidence presented so far outlines key themes explored to date around the immunoregulatory mechanisms of FMT in IBD, as summarized in Fig. 1. Administration of FMT is associated with an increase in the production of specific anti-microbial peptides, secretory IgA and mucin, thereby minimizing pathogen invasion by antigen/pathogen-dependent and -independent targeting. There is some evidence to suggest a reduction of neutrophils, macrophages, proinflammatory cytokines and down-regulation of MHC-II-dependent presentation of bacterial antigens in response to FMT administration. Collectively, however, the strongest evidence, primarily from animal models and clinical studies, indicate its significance in the induction of colonic mucosal T_{regs} with an IL-10-dependent resolution of inflammation in IBD. This amelioration of colonic inflammation was associated with enrichment of specific *Clostridium* clusters that include the SCFA-producing families *Ruminococcaceae* and *Lachnospiraceae* and genus *Roseburia*.

An increasingly recognized feature of gut commensals is their ability to induce T_{regs} via various molecular mechanisms (Fig. 2). T_{regs} play a critical role towards maintaining peripheral tolerance *in vivo* by actively suppressing pro-inflammatory T cell cytokine secretion and proliferation. Thus, these cells are critical towards maintaining immune balance and restricting aberrant inflammation. It is likely that introduction or enrichment of specific strains of bacteria through FMT promotes T_{reg} proliferation in the colonic mucosa and lymph nodes, thereby attenuating inflammation [111]. T_{regs} can be induced through products of bacterial metabolism, including SCFA, tryptophan metabolites and PSA.

SCFA such as acetate (C2), propionate (C3) and butyrate (C4) and organic acids (lactate) are derived from the fermentation of host-indigestible polysaccharide and oligosaccharide, together with certain amino acids [112]. The metabolism of these saccharides involves enzymatic pathways that are facilitated by several bacterial species. The transcription factor FoxP3 is centrally involved in the establishment and maintenance of the T_{reg} cell phenotype, and its activity is regulated post-translationally by histone/protein acetyltransferases and histone/protein deacetylases (HDACs) [113]. SCFAs produced by these bacteria are involved in stimulating colonic T_{regs} through direct physical inhibition of HDACs or inhibition of HDACs through specific G-protein-coupled receptors signalling, including G protein-coupled receptors (GPR)41, GPR43 and GPR109a [114]. Furthermore, SCFA promotes differentiation of naive

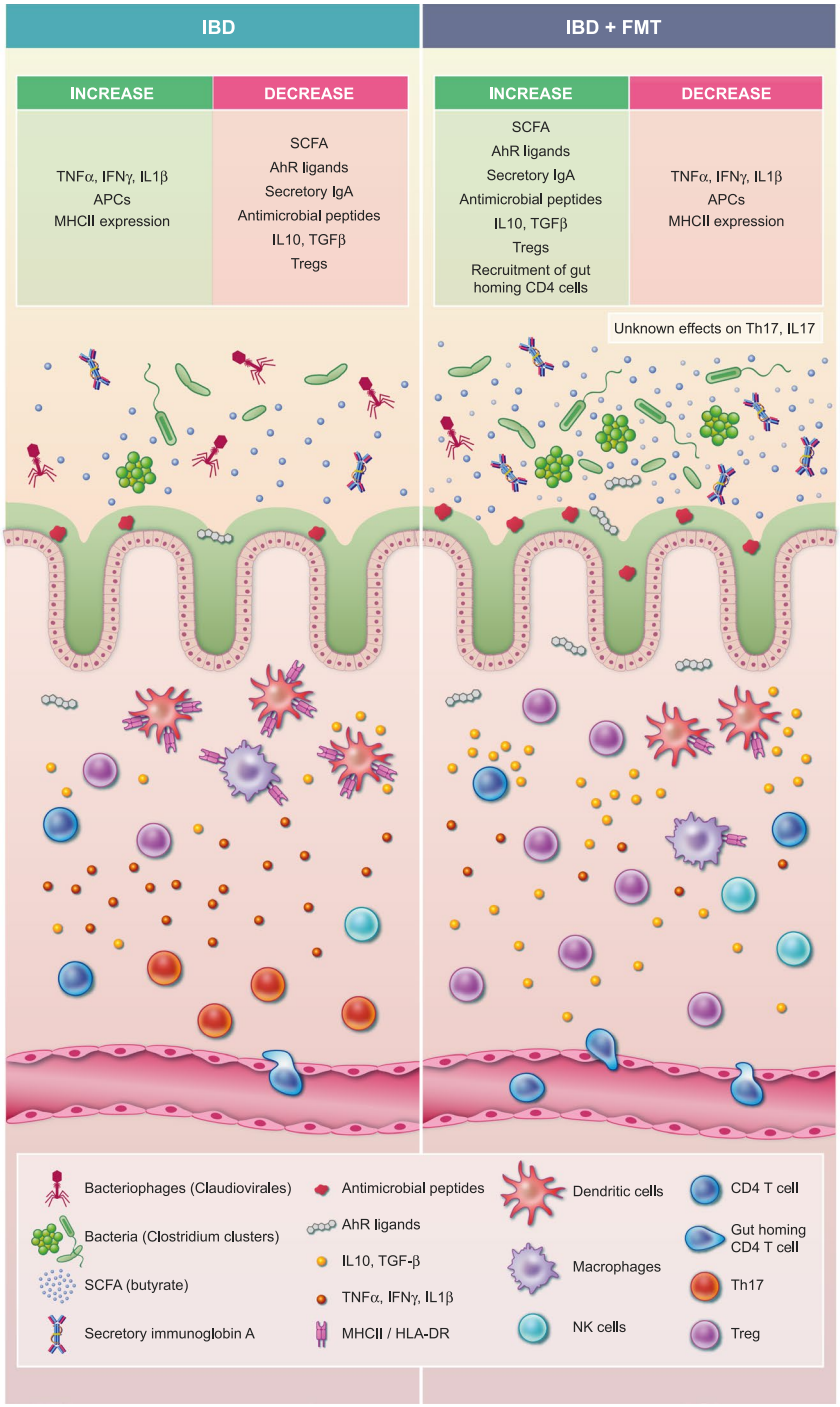

Fig. 1. Illustrative summary of evidence to date demonstrating intestinal mucosal immunological changes associated with faecal microbiota transplantation (FMT). FMT is associated with an increase in production of anti-microbial peptides, including cathelicidin anti-microbial peptide (Camp), S100A8, specific defensins, secretory immunoglobulin A and mucin. Reduction of antigen-presenting cells, including neutrophils and macrophages and up-regulation of regulatory T cells (T_{regs}), interleukin (IL)-10-secreting CD4 T cells and circulating gut-homing CD4 T cells following FMT. Consequently, there is an increase in IL-10 and transforming growth factor (TGF)-β production and a reduction of proinflammatory cytokines, including tumour necrosis factor (TNF)-α, interferon (IFN)-γ and IL-1β. A down-regulation of major histocompatibility complex (MHC)-II dependent presentation of bacterial antigens via dendritic cells is also noted following administration of FMT. These findings are associated with amelioration of intestinal inflammation. Administration of FMT is associated with enrichment of specific *Clostridium* clusters that include the short chain fatty acids (SCFA)-producing families *Ruminococcaceae* and *Lachnospiraceae* and genus *Roseburia* in clinical studies.

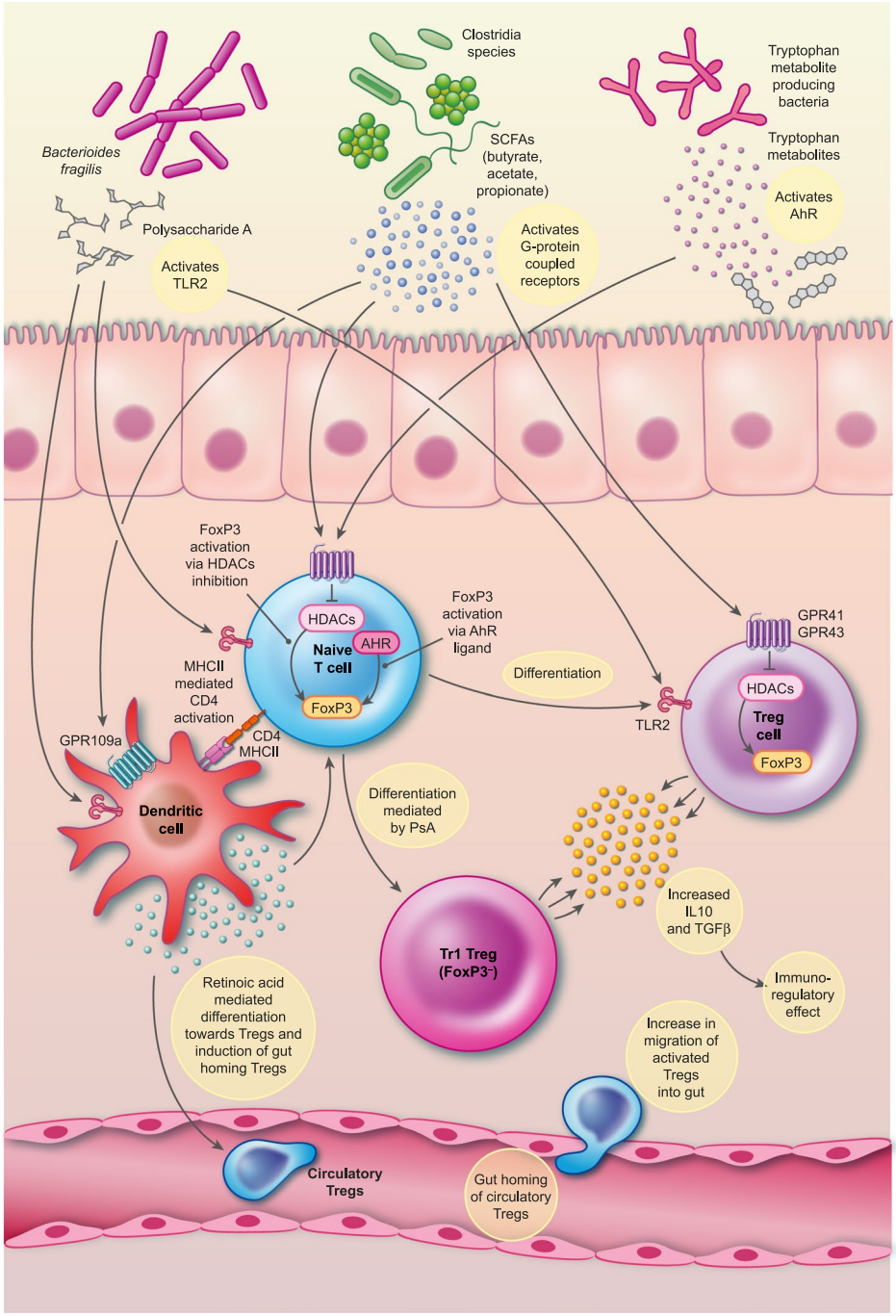

Fig. 2. Probable immunoregulatory mechanisms mediated by gut microbiota as a consequence of faecal microbiota transplantation (FMT) in inflammatory bowel disease (IBD). Microbiota-derived enzymatic activities generate metabolites such as short chain fatty acids (SCFA) and kynurenines. SCFA is involved in stimulating colonic regulatory T cells (T_{regs}) and differentiation of naive CD4 T cells through inhibition of histone/protein deacetylases (HDACs) via specific G-protein-coupled receptors signalling. This results in increased histone acetylation in the forkhead box protein 3 (FoxP3) gene together with an increased FoxP3 expression. Kynurenines (tryptophan metabolites) activate the AhR pathway to polarize naive T cells into FoxP3$^+$ T_{regs}. *Bacteroides fragilis*-derived polysaccharide A (PSA), induces differentiation of naive CD4 T cells towards interleukin (IL)-10-producing T_{regs} or into type 1 (Tr1) regulatory T cells T_{regs} (FoxP3$^-$) via plasmacytoid dendritic cell stimulation. Additionally, an increase in gut-homing of IL-10-producing T_{reg} cells and differentiation of T_{regs} is induced by retinoic acid generated by dendritic cells in the presence of microbial antigens.

CD4 T cells towards a FoxP3 CD4 cell phenotype. Murine and cell culture studies have demonstrated the direct effect of SCFAs and SCFA-producing bacterial species in inducing intestinal T_{regs} specifically, with higher levels of FoxP3 and IL-10 expression [115,116]. Additionally, SCFAs suppress the LPS and cytokine-stimulated production of pro-inflammatory mediators, including TNF-α, IL-6 and nitric oxide (NO) [117].

As described earlier, the gut microbiota–butyrate–T_{reg} axis has been explored in seminal work conducted by Atarashi and colleagues [67,68]. They found that a consortia of 46 *Clostridium* species was able to significantly raise the baseline depressed levels of regulatory T cell populations in GF mice to the levels observed in conventionally raised mice. Interestingly, the T_{regs} induced by these *Clostridium* species were Helios-negative IL-10-expressing iT_{regs}, suggesting peripheral rather than thymic induction of these immunoregulatory cells. Although they demonstrated that T_{reg} differentiation was directly influenced by epithelial cell-derived TGF-β1 and indoleamine 2,3-dioxygenase, specific microbe-associated molecular patterns were not identified. Furthermore, oral inoculation of *Clostridium* of conventionally raised mice during early life resulted in resistance to colitis. Furusawa and colleagues subsequently demonstrated that colonic luminal concentrations of SCFA positively correlated with colonic T_{reg} abundances [118]. The differentiation of T_{reg} cells, both *in vitro* and *in vivo*, was induced by butrytate and ameliorated the development of colitis induced in an adoptive T cell transfer model of $Rag1^{(-/-)}$ mice. They identified enhanced histone H3 acetylation in the promoter and conserved non-coding sequence regions of the FoxP3 locus of naive T cells in the presence of butyrate, suggesting a possible mechanism for microbial-derived butyrate-induced differentiation of T_{regs}. These findings, together with microbiota outcomes from studies of FMT in UC, highlight the role of commensal *Clostridia* as being leading players in the maintenance of gut homeostasis. Phase 1 clinical studies towards exploring the efficacy of human commensal *Clostridia* strains for the treatment of IBD are currently in progress [119].

PSA, a glycoantigen derived from *Bacteroides fragilis*, has been shown to induce IL-10-producing T_{regs} via plasmacytoid dendritic cell stimulation in the gut [120,121]. In the absence of APCs, this pathway is also mediated by direct interaction of PSA with TLR-2 expressed by $FoxP3^+$ T_{reg} cells to stimulate expression of immunoregulatory molecules, including IL-10, TGF-β2 and granzyme B [122]. Additionally, exposure to PSA induced differentiation of human peripheral $CD4^+$ T cells into type-Tr1 T_{regs} ($FoxP3^-$) and exhibited non-specific IL-10-mediated bystander suppression. Intriguingly, glycoantigen exposure provoked expression of gut-homing receptors on their surface to facilitate gut localization of anti-inflammatory and regulatory responses [123]. Furthermore, gut-homing of IL-10-producing regulatory T cells and differentiation of naive CD4 T cells towards a T_{reg} phenotype is induced by interaction of vitamin A metabolite all-*trans* retinoic acid generated by dendritic cells in the presence of microbial antigens [124]. However, the underlying mechanisms dictated by gut microbiota in modulating intestinal retinoic acid is unclear. Peroxisome proliferator-activated receptor gamma (PPARγ) activation has been shown to drive a T_{reg} phenotype [125]. The gut commensal *B. thetaiotaomicron* exerts its anti-inflammatory effects by regulating the nuclear-cytoplasmic shuttling of PPAR-γ, thereby influencing the immune regulatory landscape in the intestine [126]. Finally, microbial tryptophan metabolites (kynurenines) produced by bacteria such as *B. longum*, *B. thetaiotaomicron* and *F. prausnitzii* activate the AhR pathway to reprogramme intraepithelial $CD4^+$ T helper cells into immunoregulatory T cells and inhibit polarization towards a proinflammatory Th17 phenotype [127–129].

Recommendations for future research exploring use of FMT in IBD

Clinical studies investigating the therapeutic use of FMT in the treatment of IBD should now adopt a focused mechanistic approach towards exploring changes in mucosal immunology. This includes understanding immune cell subset dynamics together with exploration of inter-relationships within the spatial context of the tissue micro-enviroment. As immune cell yield obtained from colonic biopsies limits detailed immunophenotyping via flow cytometry, techniques such as mass cytometry may be more appropriate. Furthermore, single cell sequencing of immune subsets, in particular T cells, would help to delineate tissue-specific adaptation, transcriptional dynamics and plasticity potentially towards a T_{reg} phenotype in response to FMT. Finally, undertaking a systems biology host-microbial 'omics' integrative approach has the clear potential of identifying microbiota-derived mediators in driving immunoregulatory pathways.

Conclusions

Accumulating evidence supports the causal contribution and centrality of an interaction of a disrupted gut microbiota with a primed immune response in setting up a proinflammatory environment that leads to the development and progression of IBD. Modulation of this dysbiosis by FMT shows promise in the treatment of IBD; however, strong data regarding the biological basis of this success are lacking. Insights from clinical studies and animal experiments suggest both association and a direct contribution of changes in specific members of the gut microbial community as a consequence of FMT that dictate a shift in

homeostatic balance towards intestinal immunoregulatory pathways. Further careful mechanistic exploration from human FMT studies with the aim of exploring important interactions between the microbiome and the immune system will uncover potential targets for intervention and, equally importantly, the microbial triggers for IBD.

Author contributions

M. N. Q. and W. S. performed a literature review and wrote the review. M. N. Q. prepared the figures with input from all authors. All authors read, edited, and approved the final draft.

Disclosures

All authors have no conflicts of interest to declare.

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