

# Mechanisms underpinning the efficacy of faecal microbiota transplantation in treating gastrointestinal disease

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**Abstract:** Faecal microbiota transplantation (FMT) is currently a recommended therapy for recurrent/refractory *Clostridioides difficile* infection (CDI). The success of FMT for CDI has led to interest in its therapeutic potential in many other disorders. The mechanisms that underpin the efficacy of FMT are not fully understood. Importantly, FMT remains a crucial treatment in managing CDI and understanding the mechanisms that underpin its success will be critical to improve its clinical efficacy, safety and usability. Furthermore, a deeper understanding of this may allow us to expose FMT's full potential as a therapeutic tool for other disease states. This review will explore the current understanding of the mechanisms underlying the efficacy of FMT across a variety of diseases.

**Keywords:** faecal microbiota transplantation, gastrointestinal disease, mechanistic, metatranscriptomics, microbiota

Received: 7 June 2020; revised manuscript accepted: 13 July 2020.

## Introduction

Faecal microbiota transplantation (FMT) is currently a recommended therapy for recurrent/refractory *Clostridioides difficile* infection (CDI).<sup>1–4</sup> It is also being explored in the research setting for many other indications.<sup>5</sup> However, there are a number of associated concerns regarding its use, including the unpleasant prospect of the procedure, the potential need for invasive administration,<sup>6</sup> the small, but recognised risk of transmission of infection, and the complex regulation associated with its use.<sup>7</sup> The COVID-19 pandemic and potential risk of viral transmission through donor stool samples has brought its limitations to the fore.<sup>8</sup> As such, from a therapeutic perspective, understanding the mechanisms that underpin the efficacy of FMT may enable us to refine FMT from its current relatively crude state to a more refined 'microbiome therapeutic', which is no longer FMT, but could have a greater overall safety profile. This review will explore the current understanding of the mechanisms that underpin the efficacy of FMT across a variety of diseases.

## Current indications for FMT

There has been a wealth of evidence demonstrating that FMT for CDI is effective for recurrent and refractory CDI, and the treatment has therefore been adopted in national and international guidelines.<sup>2–4</sup> A meta-analysis of all these studies highlights clinical resolution in 92% (95% CI 89–94%) of cases.<sup>1</sup> The success of FMT for CDI has led to interest in its therapeutic potential in many other disorders,<sup>7,9</sup> but a report on these is beyond the scope of this review.

## Constituents in FMT that are associated with response

### Microbial alterations

In CDI, the suppression of the native gut microbiome, often by antibiotic treatment, enables *C. difficile* spores to germinate into vegetative cells, which produce enterotoxins that cause inflammation and result in debilitating diarrhoeal symptoms.<sup>10</sup>

*Ther Adv Gastroenterol*

2020, Vol. 13: 1–14

DOI: 10.1177/  
1756284820946904

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The key rationale for using FMT as treatment for CDI is that this therapy restores the gut microbial communities. Indeed, the ‘healthy commensals’ reintroduced through FMT will compete for the ecological niches and prevent colonisation by pathogens, a well-described phenomenon known as ‘colonisation resistance’. The role of the gut microbiota as a factor in the pathogenesis of many conditions including inflammatory bowel disease (IBD), metabolic syndrome and subgroups of patients with irritable bowel syndrome (IBS)<sup>7</sup> is accepted. However, the relative importance of the gut microbiota in the overall pathogenesis is different from one disease to another and we cannot yet quantify it for many diseases. For example, it has been noted that in CDI, changes in the composition of the gut microbiota represents the predominant factor in CDI pathogenesis<sup>11</sup> and, in IBD, it plays a very important role.<sup>12</sup> For many other conditions, its role might be more limited compared with other factors.

Furthermore, other than for CDI, mechanistic studies are largely lacking, and it remains overall unclear whether these microbiota changes play a role significant enough to be efficiently targeted by FMT or other microbiome-based intervention.

More recent data have shown that the efficacy of FMT in the treatment of recurrent CDI (rCDI) may not be explained by purely restoration of gut bacteria *per se*, but also by a number of additional factors. For instance, in one pilot study, researchers prepared a sterile faecal filtrate by passing FMT slurry through progressively narrower pore filters, culminating in a 0.2 µm pore filter.<sup>13</sup> The administration of the sterile faecal filtrate *via* a nasojejunal tube was effective in treating five patients with rCDI (>6 months), comparable with that degree of efficacy seen after administration of conventional FMT. The authors concluded that, rather than FMT directly requiring live, intact bacteria for its efficacy, it was instead likely that one or more soluble factors associated with bacteria within the filtrate potentially mediated its mechanism of action.<sup>13</sup> Within the following sections, the potential contributions of such factors are discussed.

#### Bacteriophage alterations

Bacteriophages are viruses that target and replicate within bacteria or archaea.<sup>14</sup> Importantly, phage exposure can alter both the virulence and

biofilm of its host.<sup>15</sup> From FMT/CDI studies, it has been shown that abundance of the order of bacteriophages named *Caudovirales* reduced significantly in stool after FMT, with FMT success more likely if donors had a higher fraction of *Caudovirales* within their stool virome.<sup>16</sup> Following FMT for rCDI, a recipient’s core virome quickly resembled that of a donor and remained stable over at least the next 7-month period and even up to 12 months.<sup>17,18</sup> In terms of other diseases, there are controversial data regarding phages, but successful FMT for IBD was associated with low eukaryotic viral richness in recipients before FMT.<sup>19</sup> Further supporting the role of the virome was a recent mouse study that transferred lean faecal virome into mice fed with high-fat diet. The virome transfer led to reduced weight gain and normalised blood-glucose relative-control mice. The authors concluded that the faecal virome exerts its effects *via* changes in the gut microbiota.<sup>20</sup>

Importantly, eukaryotic viruses can be found in food and hence diet could be a confounding factor.<sup>21</sup> However, it is likely that the gut virome plays a significant part by its interaction with the other components of the gut microbiota,<sup>15</sup> but from a mechanistic perspective there are limited data to explain how bacteriophages and the virome contribute to a successful FMT and at present, we are limited to associative studies, and hence studies that infer causation are needed. It is likely that bacteriophages can alter their bacterial hosts indirectly by reprogramming their metabolism, to include transfer of phage genes that encode for antibiotic resistance<sup>22</sup> and alterations in pathogen virulence.<sup>23</sup> It is therefore likely that the enteric virome may contribute to some of the mechanisms that underpin the success of FMT, but this requires further exploration.

#### Mycobiota alterations

From a fungal perspective, it has been suggested that patients with CDI who responded to FMT experienced colonisation with particular donor-derived fungal taxa (in particular, members of *Saccharomyces* and *Aspergillus* genera), whereas non-response was associated with a dominant presence of *Candida* within donor stool.<sup>24</sup> Individuals not responding to FMT and/or patients treated for rCDI with antimicrobials alone retained overgrowth of *Candida*. In a mouse model of CDI, the presence of *Candida albicans*

was associated with reduced efficacy of FMT, while use of antifungal therapy helped restore efficacy.<sup>24</sup> Utilising internal transcribed spacer 2 (ITS2) sequencing, it was demonstrated that the fungal microbiota is skewed in IBD, with an increased Basidiomycota/Ascomycota ratio, a decreased proportion of *Saccharomyces cerevisiae* and an increased proportion of *C. albicans* compared with healthy controls.<sup>25</sup> In samples from a large randomised controlled study utilising FMT for ulcerative colitis (UC) it was found that high *Candida* abundance pre-FMT was associated with a clinical response, whereas decreased *Candida* abundance post-FMT was indicative of ameliorated disease severity.<sup>26</sup> The authors suggested that high *Candida* abundance in the recipient might promote the engraftment of donor's bacteria by freeing ecological niches, and that FMT may reduce *Candida* which culminates in the success of FMT. These potential mechanisms need further exploration while acknowledging caveats associated with fungal infections in the presence of immunosuppression.

Importantly, trans-kingdom-fungi–bacteria interactions have good evidence in many ecosystems and are beginning to be further understood in the gut.<sup>27</sup> While the perturbation of gut fungal profiles and their influence upon FMT outcomes are of interest, their significance as potentially contributing to the efficacy of FMT remains unclear. In view of the established relationship between antimicrobial treatment and overgrowth of *Candida* within the gut, any changes in gut mycobiota profiles may only possibly be proxies of gut bacterial alterations. As such, the specific contribution of bacteriophages and fungi to the efficacy of FMT remains undefined.

### Metabonomics

Metabonomics is defined as ‘the quantitative measurement over time of the metabolic responses of an individual or population to drug treatment or other intervention’; this differs from metabolomics, that explore the metabolic responses present in the whole cell or tissue.<sup>28</sup> Metabonomics, therefore, explore responses of an individual or community, whereas metabolomics explore responses in a cell, or bacterial population. Metabonomics utilise integrated-systems biology to provide a way of investigating the metabolic status of an organism or ecosystem by studying ‘real’ metabolic endpoints.<sup>29</sup> The contribution of

gut microbiota-derived metabolites, or ‘co-metabolites’ produced through the interaction between the microbiota and host, has also been a key area of interest in the study of mechanisms of FMT.

*Short-chain fatty acids.* One particular group of metabolites that have been well studied in this field include short-chain fatty acids (SCFAs), which are the products of bacterial fermentation of partially digestible and non-digestible dietary carbohydrates and amino acids. Mice treated with broad-spectrum antibiotics experienced a marked reduction in levels in SCFAs in stool, and higher SCFA levels correlated with protection from *C. difficile* growth, suggesting an interaction between antibiotics, SCFAs, and CDI risk.<sup>30</sup> More recent work used a bioreactor/chemostat model of CDI to demonstrate that cessation of broad-spectrum antibiotics was associated with spontaneous recovery of the microbial synthetic recovery of most SCFAs, but not that of valerate, the five-carbon SCFA.<sup>31</sup> *In vitro*, valerate caused a dose-dependent inhibition of the growth of a range of *C. difficile* ribotypes, without any adverse effects against any commensal bacteria. Furthermore, in a mouse model of CDI, oral gavage of glycerol trivalerate was demonstrated to cause a rapid reduction in *C. difficile* colony-forming units detectable within stool.<sup>31</sup> Further experiments demonstrated that successful FMT for rCDI in humans was associated with the rapid, sustained restoration of stool valerate levels.<sup>31</sup> Beyond the dominant SCFAs of acetate, butyrate and propionate, these data support a specific role of valerate recovery in the success of FMT for rCDI.<sup>32,33</sup>

Furthermore, SCFAs seem to be critical in driving intestinal homeostasis through immunometabolic pathways in IBD.<sup>34</sup> SCFAs, specifically butyrate, have been shown to promote regulatory T-cell response in murine models of IBD.<sup>35</sup> Gut microbiota analysis of FMT-treated mice showed significant increases of commensals, including members of Lactobacillaceae and streptococcus along with of the SCFA-producing taxa Erysipelotrichaceae and Ruminococcaceae.<sup>36</sup> Administration of FMT is associated with enrichment of specific clostridium clusters that include the SCFA-producing families Ruminococcaceae and Lachnospiraceae and genus Roseburia in clinical studies.<sup>36</sup> Taken together, these findings suggest that restoration of gut microbial SCFA producers through FMT may drive regulatory

immunological responses and homeostatic balance in IBD.

**Bile acids.** A further group of metabolites of particular interest in the field of FMT/CDI is the bile acids. Initial experiments *in vitro* over 10 years ago demonstrated the differential effects of different classes of bile acids upon *C. difficile*. Specifically, primary bile acids have a ‘pro-*C. difficile*’ effect, primarily through the promotion of spore germination; in particular, the conjugated bile acid taurocholic acid (TCA) strongly promotes *C. difficile* germination *in vitro* in the presence of glycine as co-germinant.<sup>37,38</sup> Conversely, the secondary bile acids (including deoxycholic and lithocholic acid) have a net ‘anti-*C. difficile*’ effect, particularly through the inhibition of vegetative growth and toxin activity of the bacterium.<sup>30,37</sup> The transition from primary to secondary bile acids within the gut occurs through the activity of enzymes produced by the gut microbiota (in particular, the enzymes bile salt hydrolase (BSH) and 7- $\alpha$ -dehydroxylase). Rodent studies supported the concept that restoration of bacterial bile-metabolising capacity to the gut microbiota was protective against CDI,<sup>39</sup> prompting interest into whether this could also be a mechanism of efficacy of FMT. In this context, a range of *in vitro*, rodent and human studies have collectively demonstrated that while the pre-FMT stool bile-acid milieu is enriched with primary bile acids (and particularly TCA), the post-FMT stool bile-acid pool is much more comparable with that of healthy donors, with high levels of secondary bile acids.<sup>32,33,40,41</sup> More recent work has directly demonstrated that successful FMT in those with rCDI results in maintained restoration of microbial BSH functionality to the gut microbiota, and that restoration of BSH in a mouse model of CDI is sufficient to significantly reduce *C. difficile* counts within stool.<sup>41</sup> Further research in this area has demonstrated that successful FMT for CDI is associated with an increase in circulating fibroblast growth factor (FGF)-19 and reduction in FGF-21, consistent with upregulation of the bile-acid receptor farnesoid X receptor (FXR)-FGF pathway.<sup>42</sup> An additional surprising finding of interest has been the recent demonstration that bacteria with 7- $\alpha$ -dehydroxylase bile-metabolising activity (including *Clostridium scindens*) are also able to produce tryptophan-derived antibiotics which inhibit the cell division of *C. difficile*.<sup>43</sup>

While evaluation of the effect of FMT for rCDI upon SCFAs and bile-acid metabolism has focused on their direct effects upon the life cycle of *C. difficile*, it is possible that this may also have other beneficial effects. For instance, FMT-mediated changes in bile-acid–FXR interactions may directly impact upon the colitis caused by *C. difficile*; administration of an FXR agonist in a mouse model of colitis resulted in significantly reduced colonic inflammation and a more intact intestinal barrier,<sup>44</sup> while microbially mediated production of particular secondary bile acids exhibit anti-inflammatory effects on intestinal epithelial cells<sup>45</sup> and have been recently recognised as promoting generation of peripheral regulatory T cells.<sup>46</sup> SCFAs have also been demonstrated as able to regulate the size and function of the colonic regulatory T-cell population, which was directly shown to be a protective mechanism against the development of colitis in mice.<sup>47</sup>

**Other metabolites.** A further related area of interest relates to the ability of *C. difficile* to ‘scavenge’ for metabolites within the antibiotic-treated gut as energy sources to facilitate growth. In particular, after antibiotic treatment, the loss of bacteria that compete with *C. difficile* for metabolites including the amino acid proline,<sup>48</sup> the organic acid succinate,<sup>49</sup> the monosaccharide sialic acid (derived from intestinal mucus)<sup>50</sup> and dietary trehalose<sup>51</sup> allows *C. difficile* to scavenge these metabolites unopposed, and exploit them for its growth and division. As such, it may be hypothesised that a further mechanism by which FMT functions is by restoring microbial competition within the gut, and therefore minimising an ecological niche that *C. difficile* deploys to derive energy sources.

Metabonomics have also been applied to explore mechanisms underlying the efficacy of FMT in treating UC. An experimental model in rodents found that FMT given from dextran sulfate sodium-induced UC rats to healthy rats induced UC-like changes.<sup>52</sup> It was also found that FMT from healthy rats to colitic rats induced remission. When exploring the metabonomic changes associated with this remission, it was observed that urinary hippuric acid was significantly reduced in the UC group compared with normal rats. Specifically, it was noted that there were increases in C10:3 acylcarnitine, hydroxyphenylpropionylglycine, and riboflavin. In a second experiment, researchers transferred the microbiota from those rats with

UC to untouched rats. It was noted that hippuric acid decreased in the normal rats but was restored to normal levels at day 6 and 7, and further found that the changes induced by FMT correlated with the genera *Oscillospira* and *Dehalobacterium* and the families Bacillaceae and Exiguobacteraceae.<sup>52</sup> Importantly it has been shown that hippuric acid is reduced in CD and UC due to gut microbial metabolism; this therefore suggests that FMT can alter the gut microbiome to change the metabolic drivers of disease states.<sup>53</sup>

Further supporting this concept was a study on pigs, where it was noted that FMT resulted in significant increases of the typical microbiota-derived tryptophan catabolite indole-3-acetic acid in the colonic lumen,<sup>54</sup> suggesting that tryptophan metabolites may be important actors in the efficacy of FMT.

In a human study, exploring FMT for children with UC, responders to FMT highlighted that Bacilli and Betaproteobacteria were positively correlated with metabolites from the 'disease-associated' cluster (such as creatinine and norvaline), and clostridia were positively correlated with metabolites from the 'healthy' cluster (such as xanthine and 1-hexadecanol).<sup>55</sup>

There has been one randomised controlled trial (RCT) in UC that measured metabolites following FMT using metabonomic and shotgun metagenomics. They noted that specific bacterial functional pathways were associated with a positive outcome, including: benzoate degradation, glycerophospholipid metabolism, secondary bile-acid biosynthesis, guanosine pentatetra-phosphate biosynthesis, pyruvate fermentation to acetate and lactate, biosynthesis of ansamycins, and starch degradation. Furthermore, it was found that these pathways were correlated with the abundance of *Eubacterium*, *Ruminococcus*, *Lachnospiraceae*, *Roseburia*, *Dorea* and *Coprococcus* taxa.<sup>56</sup> Linking metabolic function to specific bacteria is likely to provide key mechanistic insights into the active components in FMT and potentially help refine FMT.

*FMT metabolites and autophagy.* Autophagy is a crucial housekeeping process in cellular function that removes and recycles dysfunctional components such as misfolded proteins or damaged organelles. This process is particularly active and important for the function of proliferating cells

such as intestinal epithelial cells. It has been noted that FMT could trigger intestinal mucosal autophagy and alleviate gut-barrier injury caused by specific bacteria such as *Escherichia coli*.<sup>57</sup> Specifically, it was noted that 58 metabolites, such as lactic acid and succinic acid, were enhanced and upregulated in piglets, following FMT. These upregulations were then responsible for changes in metabolic pathways such as linoleic acid metabolism, which culminated in a decrease in intestinal permeability and enhancement of mucins and mucosal expression of tight junction proteins in the recipient.<sup>57</sup> It is therefore possible that FMT alters autophagy through its influence on the gut microbiomes metabolic pathways.

#### *Immunological mechanisms of FMT*

Through a complex and bidirectional relationship, the gut microbiome plays a critical role in shaping the gut mucosal immune response.<sup>58</sup> Our initial insight of how FMT impacted the immune system was from CDI-FMT studies.<sup>59</sup> In a dextran sodium sulfate (DSS)-induced colitis mouse model, it was noted that response to FMT was associated with activation of a variety of immune-mediated pathways which ultimately lead to interleukin 10 (IL-10) production by innate and adaptive immune cells. These included CD4+ T cells, invariant natural killer T (iNKT) cells and antigen-presenting cells. Furthermore, it was demonstrated that FMT reduces the ability of dendritic cells, monocytes and macrophages to present major histocompatibility complex class-II-dependent bacterial antigens to colonic T cells.<sup>60</sup> It has also been shown that patients with recurrent CDI who responded to FMT had a reduction in complexity serum *N*-glycosylation profiles.<sup>61</sup> Glycans are associated with epigenetic modification that affects multiple immunological pathways and enable cross talk between gut bacteria/pathogens and host epithelial cells. The relevance of this molecular mechanism in relation to response to FMT deserves further exploration.

A breakdown in the innate and adaptive immune mechanisms appears to be fundamental in the development of chronic immune-mediated diseases such as IBD.<sup>62</sup> There is now increasing evidence to suggest that the gut microbial perturbations observed in these diseases contribute to (or possibly even trigger) this homeostatic immunological imbalance.<sup>63</sup>

Transfer of gut microbiota from patients with IBD into germ-free mice has been shown to significantly increase the numbers of pro-inflammatory intestinal T-helper 17 (Th17) cells and while reducing regulatory ROR $\gamma$ t+ T-regulatory-cell (Treg) populations when compared with gut microbiota from healthy individuals.<sup>64</sup> Moreover, microbiota from patients with IBD exacerbate colitis in an immunological mouse model of IBD with correlations observed between proportions of Th17 and ROR $\gamma$ t+ Treg cells and patient inflammatory status.

The majority of mechanistic work incorporated into the five RCTs in IBD<sup>36,65–68</sup> focused on shifts in gut bacterial and metabolomic profiles, with only one exploring immunological effects of FMT on disease response. This study did not find any significant change in proportions of  $\gamma\delta$  T cells, NK cells and T-cell subsets in colonic lamina propria immune cells.<sup>65</sup> They did, however, observe a slight increase in peripheral blood mononuclear gut-homing CD4 T-cell populations following FMT when adjusted for clinical disease-activity scores ( $p=0.05$ ). It was unclear if responders to FMT had specific shifts in immune subsets compared with non-responders.

Our group (Quraishi and Iqbal) recently evaluated the host mechanistic response to FMT in patients with active UC as part of the pilot phase of the STOP-Colitis trial.<sup>69,70</sup> In the 12 patients enrolled into this mechanistic arm, a clinical response was seen in eight patients following FMT. The responders had a significant reduction in mucosal Th17 cells along with a significant increase in regulatory T cells, effector-memory Tregs and gut-homing Tregs. Furthermore, we observed a significant increase in IL-10-producing CD4 cells and reduction in IL-17-producing CD4-cell and CD8-cell populations in responders, following FMT. Colonic mucosal transcriptome analysis demonstrated that clinical response to FMT was associated with significant downregulation of host antimicrobial defence response, antimicrobial peptides and pro-inflammatory immune pathways. There was a significant upregulation of butyrate and propionate metabolic pathways in FMT responders.

A study in two patients with immune-checkpoint inhibitor colitis observed that immunological response after FMT was associated with an increase in FoxP3+ CD4 cells along with a

substantial reduction in the colonic mucosal CD8+ T-cell population.<sup>71</sup> There was a concomitant expansion in the population of *Bifidobacterium* species, Clostridia and Blautia. Treatment of mice with *Bifidobacterium* has been shown to ameliorate DSS-induced colitis following immune-checkpoint blockade.<sup>72</sup> This protective effect was, however, abrogated in Treg-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 Treg function. Furthermore, in a rodent study that inoculated 2-week old neonatal mice with faeces from Clostridium-associated mice, it was demonstrated that there was a significant increase in Clostridium clusters IV and XIVa in the treated mice accompanied by a significantly higher number of colonic FOXP3+ Tregs,<sup>73</sup> highlighting the potential interactions between the microbiome and the local/systemic immunity. In a follow-up study exploring this concept, researchers inoculated germ-free mice with either treated or untreated chloroform human stool and noted a significant increase in the percentage of FOXP3+ Tregs among CD4+ T cells in the colons of germ mice inoculated with untreated human faeces compared with germ-free mice.<sup>35</sup>

When applied to those with IBD, a study that used colonic lamina propria lymphocytes (LPLs) and peripheral blood lymphocytes (PBLs) from healthy individuals and those with colon cancer and IBD, demonstrated that DP8 $\alpha$  T cells exhibited a highly skewed repertoire toward the recognition of *Faecalibacterium prausnitzii*, which is decreased in patients with IBD. They further demonstrated that the frequencies of DP8 $\alpha$  PBL and colonic LPL were lower in patients with IBD than in healthy donors and in the healthy mucosa of patients with colon cancer, respectively. These data together suggest that *Clostridium* species are key regulators of inflammation through their influence on the gut immune system.<sup>74</sup> A further study which stimulated cells known to respond to *F. prausnitzii* measured their production of IL-10 and their downstream cell activity. They demonstrated that the proportion of circulating CCR6+ / CXCR6+ DP8 $\alpha$  T cells was significantly reduced ( $p < 0.0001$ ) within the total population of CD3+ T cells from patients with IBD compared with patients with infectious colitis or controls.<sup>75</sup> Summarising these findings suggests that components of the gut microbiome are key regulators of immune function and significantly impact the

mechanisms that underpin gut homeostasis, and therefore, FMT may work by promotion of some of the gut homeostatic immune functions and downregulating the pro-inflammatory immune responses.

When considering specific metabolites, it has been demonstrated that SCFAs, specifically butyrate, have been shown to induce Tregs and promote anti-inflammatory IL-10 production in mice.<sup>35,73</sup> It is likely that introduction or enrichment of specific gut microbial species *via* FMT attenuates inflammation by promoting Treg proliferation in the colonic mucosa through products of bacterial metabolism including SCFAs, tryptophan and polysaccharide.<sup>76–78</sup> When exploring tryptophan specifically, it has been demonstrated that the transfer of microbiota from CARD9 mice into wild-type germ-free mice increases their susceptibility to colitis. The mechanism that appears to underpin this is the CARD9 susceptibility gene alters the gut metabolism of tryptophan into aryl hydrocarbon receptor ligands, leading to inflammation.<sup>79</sup> Importantly, this phenomenon was ameliorated by inoculation of mice with *Lactobacillus* strains capable of metabolizing tryptophan, suggesting a key link between genetics and microbiota.

## Future considerations

### *Small-bowel microbiota*

Importantly, the majority of published studies focus on the colonic microbiota, as assessed by stool (or, in some cases, by mucosal biopsies). The small bowel also harbours a complex microbial community, albeit with less diversity and abundance ( $\approx 10^3$ – $10^7$  microbial cells/g) than the colonic microbiota ( $\approx 10^{12}$  cells/g).<sup>80,81</sup> Its influence on the mechanisms and effect of FMT is currently poorly understood. Importantly, FMT is known to have comparable efficacy in CDI<sup>82</sup> when infused into the upper gastrointestinal tract. Specifically, when considering IBS, small-bowel microbiota alterations have been associated with symptoms,<sup>83</sup> and hence future studies will need to explore the role of the small-bowel microbiota in efficacy of FMT.

### *'Super donor' concept*

Data from non-CDI FMT studies such as in IBD have demonstrated that some recipients of FMT

have an exceptional response while others do not.<sup>36,65</sup> It is therefore possible that there are factors associated with both the recipient and the donor that may underpin the success of FMT. When considering donor factors, it is likely that particular components are driving the therapeutic effect from FMT and hence analysing the donor stool remains an important element in understanding the mechanisms underpinning the efficacy of FMT. Studies have speculated about what makes a 'super donor'.<sup>84</sup> The origins of a putative 'super donor' effect were from an FMT–UC study in where 'donor B' induced significant more remission than other donors. This therapeutic effect was associated with significant increases for the family Lachnospiraceae and the genus *Ruminococcus* in 'donor B' microbiota.<sup>67</sup> Such evidence lead researchers to conclude that a donor's microbiota diversity may have an influential effect on the success of FMT in IBD.<sup>85,86</sup> Furthermore, specific taxa have been associated with disease response such as, clostridium clusters IV and XIVa<sup>68,87</sup> and Ruminococcaceae and Lachnospiraceae families.<sup>67</sup> Specific bacteria-producing SCFAs such as butyrate are also suggested to be important in the efficacy of FMT.<sup>88</sup> Furthermore, as previously stated, a meta-analysis exploring the role of FMT for IBS demonstrated that FMT had no effect in IBS, following this, however, an RCT using a single 'super donor' showed a high success at reducing IBS symptoms,<sup>89</sup> suggesting that the stool donor may have significant effects on the efficacy of FMT.

Importantly, when considering CDI, most patients respond to FMT, which suggests that disease-specific factors may be driving the success rather than the donation itself. In view of this, studies exploring donor-specific factors associated with an unsuccessful response may provide valuable insight into mechanisms that underpin FMT success. Another major consideration is that diet plays a large influence on the gut microbiota and hence is likely to affect the donation and FMT efficacy.<sup>90</sup> Uncovering the dietary aspects that may influence the efficacy of FMT will be an important consideration. Lastly, it is plausible that specific constituents in an FMT will provide benefit for one person but not another. It is therefore possible that a 'one size fits all' FMT might be replaced by a more personalised FMT as our knowledge improves regarding mechanisms that underpin a successful donor. Another important consideration is studies have

not been powered to date to understand the donor characteristics associated with success. Despite these findings, however, the mechanisms underpinning the ‘super donor’ phenomenon are yet to be detailed.

### Engraftment

An important consideration is to determine what part of the FMT engrafts into the host and may be a significant factor that underpins efficacy. Currently, there is no robust definition of engraftment. A significant consideration is to understand if FMT promotes growth of suppressed host microbiome constituents or introduces new constituents into the host. Specific strain tracking may help understand this and studies are attempting to further define this.<sup>91,92</sup>

### Post-FMT era

Given this central importance of the restoration of the gut microbiota to the efficacy of FMT in the treatment of CDI, there have been a number of different approaches towards a more defined ‘narrow spectrum’ microbiota product of well-characterised bacteria as an alternative to FMT. Proof-of-concept use of ‘defined bacterial communities’ as an alternative CDI treatment has been demonstrated in bioreactor and rodent models,<sup>93,94</sup> as well as human studies. For instance, as early as 1989, bacteriotherapy was discussed in the treatment of CDI.<sup>95</sup> More recently, in the ‘RePOOPulate’ study, 33 different commensal bacterial species were cultured from the stool of healthy donors; these were used to synthesise a ‘stool-substitution therapy’, consisting of a mixture of purified bacterial cultures derived from these stool bacteria.<sup>96</sup> After colonoscopic administration of this mixture to two patients with rCDI, both patients achieved a rapid and sustained remission.<sup>96</sup> As an alternative approach, healthy donor stool was ethanol treated (to kill vegetative cells); the surviving spores were fractionated and capsulised, and delivered orally as a preparation named SER-109.<sup>97</sup> In a cohort of 30 patients, 29 achieved clinical remission from rCDI after one or two administrations of SER-109.<sup>97</sup> However, despite early promise, SER-109 produced negative results when administered in a phase II clinical trial, with potential issues related to the differentiation of true CDI recurrence from post-CDI IBS, and the dosing of the treatment regimen.<sup>98,99</sup> This concept has been further expanded

into other disease areas with a consortium of microorganisms being explored for treatment of mild-to-moderate UC in a phase II study.<sup>100</sup>

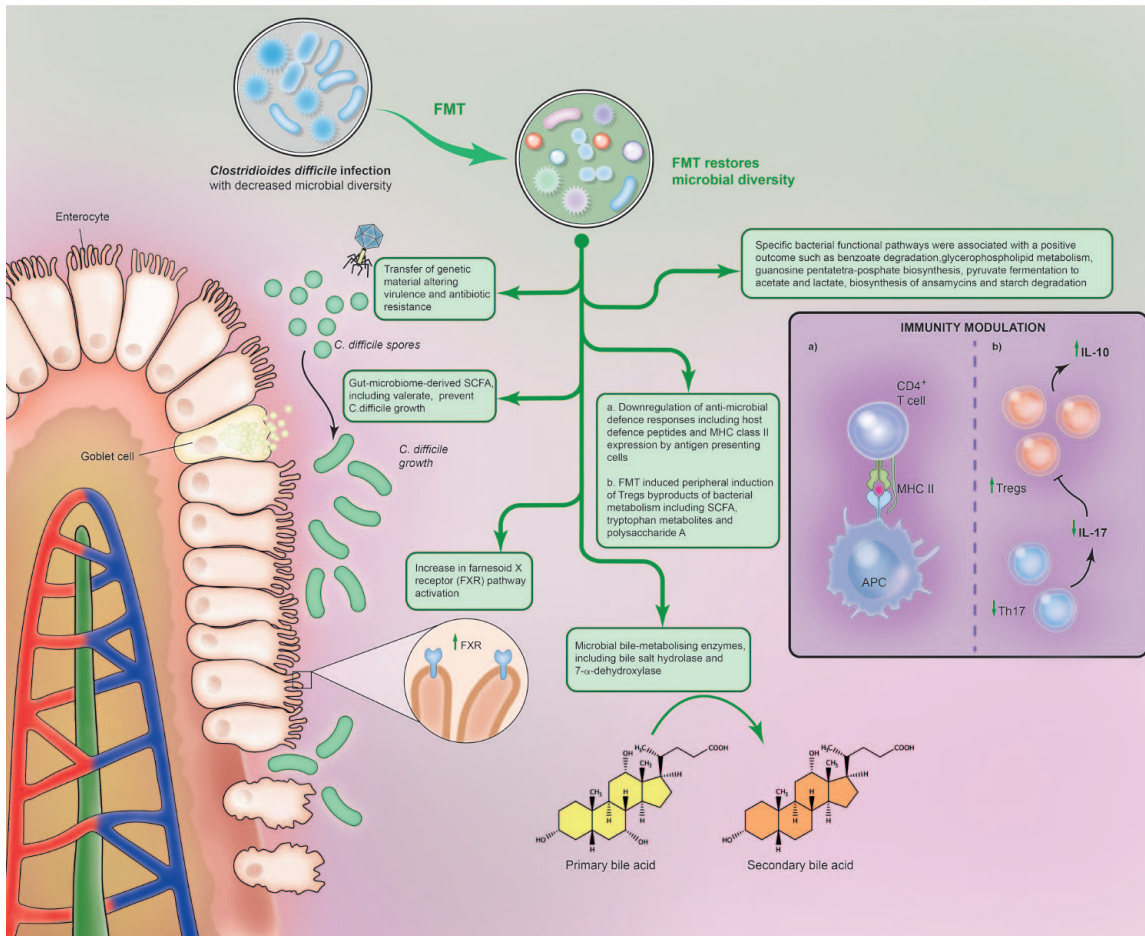
Live biotherapeutics refer to live microorganisms that are used to prevent or cure human disease.<sup>101</sup> The concept relies on specific microbes causing a beneficial effect to the host. These can be isolated from the gut microbiota of healthy people or engineered microbiomes.<sup>102</sup> As previously mentioned, these have been studied for diseases such as CDI and IBD but have shown promise in other disease areas. Specifically, they have shown promise in the treatment of cancer, with one study highlighting that a commensal of 11 healthy human-associated bacterial strains can induce interferon  $\gamma$ + CD8 T cells that confer resistance to the intracellular pathogen *Listeria monocytogenes*, and inhibit tumour growth in conjunction with immune-checkpoint inhibitors.<sup>103</sup> In another study, 17 human-derived clostridium strains (VE202) were able to reverse histological colitis in a mice model.<sup>104</sup> There are many commercial companies aiming to find biotherapeutics for a whole range of diseases. As we learn more about the mechanisms that underpin the efficacy in FMT, it is likely that these will feature in more clinical trials. Importantly, any engineered microbiota-based therapies will need to be examined in clinical trials to assess if they have clinical equipoise with, or are even superior to, FMT.

Phage therapy refers to the therapeutic use of viruses that infect bacteria, bacteriophages, to treat disease. Phage therapy aims to specifically kill their respective bacterial host while preserving other microorganisms and human cells. This has been a growing area of interest in view of the rising incidence of antibiotic resistance. Phage therapies in clinical practice are very much still in the research stage with concerns over regulation and safety.<sup>105</sup> In an *in vitro* human model study, phage  $\phi$ CD27 showed significant reduction in *C. difficile* cell numbers and toxin production without major effects on other members of the microbiota.<sup>106</sup> As previously demonstrated, the virome plays a significant role in the efficacy of FMT and hence further exploration into phage therapy may help us understand the mechanisms that underpin FMT efficacy (Figure 1).

### Conclusion

As highlighted in this review, much of our current understanding of mechanistic insights into the





**Figure 1.** Mechanisms that underpin the efficacy of FMT.

APC, antigen-presenting cell; FMT, faecal microbiota transplantation; IL, interleukin; MHC, major histocompatibility complex; SCFA, short-chain fatty acid; Th17, T-helper 17 cell; Tregs, T-regulatory cells. Illustration courtesy of Alessandro Baliani. Copyright © 2020.

efficacy of FMT are extended from what we have found from CDI studies. Certain mechanistic theories with circumstantial data to support them but no direct investigation as yet, for example, restored microbiota outcompeting *C. difficile* for the scavenging of carbon sources. Future studies should help test some of these mechanistic insights and attempt to understand the mechanisms that underpin successful FMT for specific disease indications. This may allow us to personalise FMT to not only disease states but to an individual and possibly refine FMT into a more targeted, efficacious, safer therapy.

#### Author contributions

JPS, BHM, MNQ, JM were responsible for conception, literature review, writing and revising the manuscript. TI, JRM, and HS gave critical

revisions and helped revised the manuscript. All authors agreed to the final version.

#### Conflict of interest statement

The authors declare that there is no conflict of interest.

#### Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: the Division of Digestive Diseases at Imperial College London receives financial support from the National Institute of Health Research (NIHR) Imperial Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London. BHM is the recipient of an NIHR Academic Clinical Lectureship.

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