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Understanding the mechanisms of faecal microbiota transplantation

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Abstract

This Review summarizes mechanistic investigations in faecal microbiota transplantation (FMT), which has increasingly been adapted into clinical practice as treatment for *Clostridium difficile* infection (CDI) that cannot be eliminated with antibiotics alone. Administration of healthy donor faecal microbiota in this clinical situation results in its engraftment and restoration of normal gut microbial community structure and functionality. In this Review, we consider several main mechanisms for FMT effectiveness in treatment of CDI, including direct competition of *C. difficile* with commensal microbiota delivered by FMT, restoration of secondary bile acid metabolism in the colon and repair of the gut barrier by stimulation of the mucosal immune system. Some of these mechanistic insights suggest possibilities for developing novel, next-generation CDI therapeutics. FMT might also have potential applications for non-CDI indications. The gut can become a reservoir of other potential antibiotic-resistant pathogens under pressure of antibiotic treatments, and restoration of normal microbial community structure by FMT might be a promising approach to protect against infections with these pathogens as well. Finally, FMT could be considered for multiple chronic diseases that are associated with some form of dysbiosis. However, considerable research is needed to optimize the FMT protocols for such applications before their therapeutic promise can be evaluated.

The germ theory of disease paradigm, as it was formulated in the late 19th and early 20th centuries, was centred on killing infectious pathogens with little consideration for the bystander effects on the indigenous microbial communities (microbiota) that inhabit the human body. The side effects of antibiotics, at least in the short-term, seemed to be quite favourable and many recalcitrant lethal infectious diseases became suddenly treatable. The problem of emerging antibiotic resistance, which was recognized quite early, had been largely solved by introduction of successive generations of antibiotics, which commonly had

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broader spectra of activity against a greater range of bacterial taxa[1]. Nevertheless, the problems posed by multidrug-resistant microorganisms have become one of the most urgent and growing issues in health care[2]. The increasing challenges presented by increasing failures of antibiotic treatments in clinical practice have also led to a new focus on the protective roles of the indigenous microbiota, which normally contrib utes substantially to colonization resistance against a wide range of infectious pathogens.

Clostridium difficile is one of the 'superbugs' that has grown in incidence, morbidity and mortality over the past 20 years[3,4]. This infection is typically enabled by suppression of native gut microbiota by antibiotic treatments. Upon reaching the colons of vulnerable individuals, *C. difficile* spores germinate into vegetative cells, which produce enterotoxins that cause inflammation and result in debilitating diarrhoeal symptoms[5]. Paradoxically, antibiotics also constitute the standard treatments for *C. difficile* infection (CDI), but can also perpetuate its recurrence due to progressive suppression of native gut microbiota. Chances of spontaneous relapse of the infection increase, usually at a rate of 20–30%[3,6], with each round of antibiotic treatment until some patients develop an indefinite cycle of recurrent *C. difficile* infections (R-CDI) that cannot be broken with any known antibiotic regimen[5].

Faecal microbiota transplantation (FMT) (BOX 1) normalizes the composition and functionality of gut microbiota[7–13] and has now become widely accepted as a highly successful rescue treatment for R-CDI[14]. In addition, FMT is emerging as the best option for patients with acute, severe and complicated forms of CDI that fail to respond to antibiotic treatments[15,16]; an important issue as R-CDI is associated with high mortality following current standard surgical options as treatment, which includes removal or bypass of the diseased colon[17,18].

The urgent need for alternatives to antibiotics by thousands of patients with refractory forms of CDI has led to the development of FMT as an increasingly standardized therapeutic agent. Faecal microbiota can be separated from stool of carefully selected donors, quantified in terms of viable bacteria, cryopreserved and banked[19]. The transplant material can be delivered by various routes, including an encapsulated form as an oral medication[20]. However, mechanistic understanding of how FMT actually works is needed for development of next-generation FMT-based therapeutics that can be standardized for efficacy and can be fully embraced by regulatory agencies. In addition, understanding mechanisms of FMT can inform development of more standard therapeutics, whether those might be specific molecules or highly defined microbial drugs. In this Review, we discuss the potential mechanisms that are involved in suppression and clearance of CDI following restoration of normal microbiota composition following FMT.

FMT-associated microbiota changes

The idea that FMT leads to donor-like normalization of the faecal microbial community structure derives primarily from studies including patients treated for the refractory R-CDI syndrome. Importantly, these studies have largely focused on patients who suffered multiple CDI recurrences and were exposed over many months to multiple courses of antibiotics with

broad activity against the majority of colonic bacteria. Loss of microbial diversity has been shown to be progressive in patients with CDI and proportional to the number of rounds of antibiotic treatments[21]. In our centre, for example, an average patient has experienced around five episodes of CDI over a period of 1 year before FMT[19]. Thus, it is not surprising that such patients are found to have profound dysbiosis commonly characterized by complete disappearance of Bacteroidetes, a marked reduction in Firmicutes and massive increases in the relative abundances of Proteobacteria[13]. Colonoscopic administration of FMT results in prompt normalization of the microbial community structure that closely resembles the composition in donors as early as 24 h after the procedure[12]. The speed of normalization, a marked increase in the overall microbial diversity and the restoration of the dominance of faecal bacterial composition by members of Bacteroidetes and Firmicutes phyla make it nearly certain that donor microbiota become established and engrafted into recipients of FMT with refractory R-CDI. In fact, limited investigations suggest that donorlike microbiota composition persists in these patients for months and even years[8,22].

The situation is much less clear with respect to attempts to assess engraftment of gut microbiota in conditions other than refractory R-CDI, in which the dysbiosis before the procedure is generally much less pronounced. For example, a broad pattern of dysbiosis in IBD has been noted, characterized by reduction in Bacteroidetes, reduced microbial diversity within the Firmicutes phylum and an increased proportion of Proteobacteria[23]. These observations suggested that FMT could be used therapeutically to correct these abnormalities and decrease gut inflammation. Thus far, however, these efforts have yielded largely disappointing results[24–26], although some studies have been more encouraging and suggest further need for protocol development[27]. Notably, IBD-associated dysbiosis, which is substantially milder than that observed in refractory R-CDI, might not be sufficient to enable substantial engraftment of donor microbiota using simplistic protocols modelled in CDI experience. In addition, measurements of engraftment of donor microbiota following FMT for IBD indications alone are considerably less straightforward. Although it is possible to measure overall microbial diversity and relative proportions of bacterial taxa, attribution of these changes to donor gut microbiota engraftment versus microbial composition changes due to altered level of bowel inflammation is challenging. Improvements in dysbiosis could be seen in a fraction of patients with IBD who experienced clinical improvement following FMT[24–26], but similar changes in microbiota composition can also be seen with TNF-targeted therapies and might simply be markers of reduced disease activity[28,29].

Measurements of donor gut microbiota engraftment could be enhanced with deeper metagenomic sequencing, which might enable a greater level of taxonomic discrimination than possible with the currently more routine, small-size, variable segment 16S ribosomal RNA gene amplicon-based sequencing[30]. In addition, all engraftment computations also need to take into account the highly dynamic behaviour of the gut microbiota, which requires resource-intensive analysis of multiple samples from both donors and recipients to get a more accurate comparison[12]. Indeed, the method ology to measure donor microbiota engraftment remains an area of active development. Ultimately, standardized methods are needed to assess engraftment for optimization of various FMT protocols, especially those used outside of R-CDI treatment. In fact, it might arguably be inaccurate to apply the term ‘FMT’ to procedures that merely involve administration of donor gut microbiota in a variety

of conditions, but fail to demonstrate consistent and substantial engraftment of donor gut microbiota coupled with failure to achieve clinical benefit.

The culture-independent methodologies used to study gut bacteria in the context of FMT have highlighted the importance of understanding the organizational structure and complexity of microbiota. In many respects, gut microbiota can be viewed as a microbial organ within the human body[31], which itself is made of communities of highly specialized microorganisms representing all three known domains of life — the Archaea, Bacteria and Eukarya, as well as viruses. These diverse microorganisms form intricate interactive and communicating networks organized by complex ecosystem and metabolic drivers. Thus far, however, studies have focused their attention mainly on bacteria[32], although initial investigations also demonstrated transmission of bacteriophages[29,33].

Continued research is needed to gain detailed understanding of the metabolic interdependence between the different microbial constituents, including members of non-bacterial domains of life and the host. Our current understanding of these relationships is still rudimentary. However, it is clear that the simplistic notion held by many physicians and patients that a few individual species of ‘good microorganisms’ can repair antibiotic-induced severe dysbiosis is both naive and incorrect. Despite numerous attempts, it is therefore not surprising that simple probiotics (either single strains or a few microorganisms) have largely failed in clinical trials to contribute substantive benefit in treatment of CDI[34]. Holding *C. difficile* in check and bridging full recovery of microbial community structure using defined consortia of bacteria isolated from the human gut as basic scaffolding might be one possible approach[35]. However, rational design and consistent manufacturing of such simplified microbial assemblies requires a mechanistic understanding of the different parts played by individual microorganisms present in the preparation with respect to a desired therapeutic activity or contribution to the community stability and resilience in the human gut. Ultimately, it might be also important to demonstrate that such preparations do not result in potentially stable but dysbiotic ecosystem configurations that could lead to undesired adverse effects for the long-term health of the host. This potential problem could be of lesser concern in FMT with non-selected faecal microbiota, which uses minimally manipulated microbial communities from faeces that are already optimized by co-evolution with their human hosts and tested in individual healthy donors over decades.

Underlying mechanisms for FMT

The resurgence of FMT in the past few years in clinical practice was prompted by the epidemic rise of antibiotic-refractory CDI[36,37], which remains the main indication for this treatment. This disease is also the only indication for FMT for which therapeutic efficacy is clear and undeniable, and why mechanistic studies in humans have also centred on this indication. Nevertheless, the general principles might be instructive for future research beyond treatment of *C. difficile*. Two broad, not mutually exclusive, mechanistic categor ies exist for the effectiveness of FMT that can be considered: the direct interaction of donor gut microbiota with *C. difficile* bacteria and micro biotamediated effects on host physiology and immune defences that are detrimental to *C. difficile* (FIG. 1). Gut microbiota can compete with *C. difficile* for nutritional and coloniza tion resources, interfere with its virulence

factors and directly kill *C. difficile* bacteria. The gut micro biota can also activate multiple host immune defences and constrain *C. difficile* via secondary bile acids, which can be inhibitory of *C. difficile* germination and vegetative growth. Detailed understanding of these different mechanisms is critical for development of next-generation therapeutics against CDI.

Competition with indigenous gut microbiota

The human colon provides a nutritionally rich environment for its microbiota: it is densely inhabited, being home to some 40–100 trillion bacteria[31,38]. The different resident microorganisms intensely compete with each other for nutrients and space, but also synergistically cooperate in digesting complex poly saccharides and other polymeric molecules. For example, *Bacteroides thetaiotaomicron*, a prominent, abundant commensal gut microorganism, liberates free sialic acid from mucin glycoproteins in the intestine, but cannot break it down further[39]. The free sialic acid can in turn be a nutrient for *C. difficile* because the organism possesses a sialic acid catabolic operon[39]. In fact, a mutant strain of *C. difficile* deficient in sialic acid consumption is compromised in its ability to expand in antibiotic-treated animals compared with wild-type *C. difficile*[39]. Intestinal microorganisms also liberate monomeric glucose and *N*-acetylglucosamine, which might become more accessible to *C. difficile* when indigenous microbiota are suppressed by antibiotics[40]. This syntrophic cross-feeding is common among gut microbiota. Notably, *C. difficile* is one of the first recognized autotrophic pathogens and can grow on merely carbon dioxide and hydrogen gases as sole carbon and energy sources, respectively[41]. This adaptation reduces competition with other microorganisms within narrowly differentiated nutritional niches and enables its survival in very unfavourable conditions[41]. Nevertheless, competitive niche exclusion is one plausible mech anism of FMT in treatment of CDI, and it has been the underlying principle hypothesis for the development of non-toxigenic *C. difficile* strains as therapeutics, which show promise in reducing recurrence of CDI[42]. However, the approach might be less successful with highly virulent, pathogenic, r-strategist (r-selection favours high fecundity rates and ability to disperse widely in unstable, unpredictable environments) strains of *C. difficile* characterized by greater efficiency of sporulation and growth[43].

Competition between microorganisms is not limited to access for nutritional resources. Production of bacteriocins, which are antimicrobial peptides with bactericidal or bacteriostatic activity, is a more active strategy against competitors[44]. An example of a narrow-spectrum bacteriocin that is highly effective against *C. difficile* is thuricin CD, a compound produced by *Bacillus thuringiensis* isolated from the human intestine[45]. Another example is nisin, which is a lantibiotic, a class of bacteriocins produced by many Gram-positive bacteria with strong antimicrobial activity against other Gram-positive bacteria, including *C. difficile*[46,47]. Nisinproducing strains of *Lactococcus lactis* have been isolated from human faeces[48]. Generally, lantibiotics are active in very low concentrations and contribute to extending the shelf-life of a variety of foods, including dairy products, by fermentation[49]. Interestingly, lacticin 3147 is broad-spectrum lantibiotic that can be produced by *L. lactis* strains in kefir, which might benefit some patients with R-CDI[50–52].

The role of secondary bile acid metabolism

C. difficile was first isolated from the stool of a healthy infant in 1935 and named for the difficulty of its isolation and culture[53]. Wilson reported in the early 1980s that addition of taurocholate greatly improved *C. difficile* spore germination and this primary bile acid has since been routinely used in laboratory *C. difficile* growth media[54]. Now, attention has once again been focused on the effects of bile acids on the life cycle of *C. difficile*. The spores of *C. difficile* germinate when they sense the appropriate host environment and favourable nutrient conditions[55,56]. A favourable host physicochemical environ ment is communicated to the spores by specific bile acids, and glycine can indicate nutrient availability. At least one germin ant receptor, CspC, has been identified in *C. difficile* spores that is stimulated by cholic acid class bile acids[57] (BOX 2). Activation of CspC triggers a protease-dependent cascade that activates a lytic enzyme, SleC, which is stored in the spore as a zymogen. This enzyme degrades the spore cortex, allowing rehydration of the desiccated core. Mutations in *SleC* and *CspC* inhibited *C. difficile* spore germination and decreased pathogenicity in an animal model[57,58].

Although bile acids in the cholic acid class generally stimulate germination of *C. difficile* spores, those in the chenodeoxycholic acid class generally inhibit germination of *C. difficile* spores[59]. Thus, the growth and reproductive responses of *C. difficile* depend on the combined concentration of all the bile acids present in the intestinal lumen. Colonic bile acid composition is normally dominated by secondary bile acids[60]. These secondary bile acids are generated following deconjugation of taurine and glycine, and 7α-dehydroxylation by some bacteria, yielding deoxycholic and lithocholic acids from cholic and chenodeoxycholic acids, respectively. Antibiotic treatments used to treat CDI inhibit bacteria involved in secondary bile acid metabolism, and secondary bile acids are essentially absent in patients with refractory R-CDI syndrome[13]. FMT promptly restores secondary bile acid metabolism and the total composition of bile acids in the faeces becomes donor-like[13]. Furthermore, *C. difficile* spore germination and vegetative growth are stimulated by the combinations of bile acids present in pre-FMT patients with R-CDI and inhibited by the combinations of bile acids present in faeces of post-FMT patients with R-CDI and their donors[61].

The relative concentrations of stimulatory and inhibitory bile acids present in the intestinal lumen is determined by their production rates in the liver, microbial metabolism and their differential uptake into the enterohepatic circulation[60]. The potential germinant deoxycholate is efficiently absorbed in the colon[62], which contributes to a colonic environment that is normally inhibitory to *C. difficile*. Interestingly, the pool size of inhibitory chenodeoxycholic class bile acids, relative to the cholic class bile acids, is smaller in women than in men[63]. This finding might be one potential explanation for the disproportionate fraction of female patients with R-CDI in FMT cohorts: the overall mix of bile acids in women should be more stimulatory to *C. difficile* because of the lower content of inhibitory bile acids[64–66].

The composition of bile acids might also be favourable to the growth of *C. difficile* in infants, since their ability to produce secondary bile acids develops only gradually during the first year of life[67]. In fact, approx imately one-third of infants carry *C. difficile* asympto matically up to 6 months of age, after which the carriage rate gradually decreases[68]. By 3

years of age, carriage rates reach 0–3%, similar to those seen in non-hospitalized adults[68,69]. Fortunately, clinical disease in children is rare before 12–24 months of age, perhaps due to the non-expression of the *C. difficile* toxin receptor in the infant colon[70].

Bile-acid-based approaches

The seemingly prominent role of bile acid metabolism in CDI suggests development of novel therapeutics beyond FMT. Possible directions for development include: defined, live microorganisms as probiotic drugs[71] that can mediate secondary bile acid metabolism; targeting host metabolism to diminish availability of germinant bile acids in the intestinal lumen; and development of bile acid analogues that are inhibitory to *C. difficile*.

Microorganisms involved in secondary bile acid metabolism are largely undefined. One of the key desired chemical transformations for inhibitory activity against *C. difficile* is the 7α-dehydroxylation step[72]. *Clostridium scindens*, one of several bacteria known to carry out this step, is able to inhibit CDI in a mouse model[72]. Additional microorganisms with similar capacity are likely to exist. Defined microbial-based therapeutics will potentially be developed to treat CDI in humans, although the final formulation might also require additional microorganisms to optimize therapeutic engraftment into the intestine.

Interestingly, the bile acid sequestrant cholestyramine has been, anecdotally, successfully used in the past in treatment of CDI[73]. An analogous resin colestipol was tested in combination with vancomycin in treatment of primary infection in a randomized trial[74]. That trial failed to show any benefit, and currently bile acid sequestrants are not recommended in treatment of active CDI because they can bind and neutralize the anti biotic[75]. However, it is possible that bile acid sequestrants might help to prevent recurrence of CDI rather than treat active disease. Notably, administration of bile acid sequestrants leads to a compensatory increase in the production of primary bile acids in the liver from cholesterol and negates the desired effects[76,77]. Concurrent administration of statin drugs can blunt the compensatory increase in hepatic bile acid production[78]. We have success fully treated several patients with R-CDI who had failed FMT with a combination of cholestyramine and lovastatin (A. Khoruts and M.J. Sadowsky, unpublished data). Emerging new drugs that can decrease hepatic bile acid production might have some potential utility in treatment of CDI. One example is a potent agonist of the farnesoid X receptor, obeticholic acid, which inhibits bile acid synthesis by stimulating the ileal hormone fibroblast growth factor 19 (REFS [79,80]).

Finally, bile acid analogues could be developed with enhanced inhibitory activity and favourable pharmacokinetic properties to ensure adequate and prolonged concentration in the colon. We have successfully treated a patient with refractory, recurrent *C. difficile* pouchitis with ursodeoxycholic acid (UDCA) after demonstrating that this minor secondary bile acid was inhibitory to her *C. difficile* isolate[81]. Unfortunately, UDCA is efficiently absorbed into the enterohepatic circulation and might not be applicable to the majority of patients with CDI in the colon. However, certain modified bile analogues can be made resistant to intestinal uptake and achieve high intracolonic concentrations[82]. Thus, it is highly likely that novel drugs inhibitory to *C. difficile* can be developed based on bile acid chemistry.

Immune-mediated colonization resistance

Continuous signalling from indigenous microbiota is required to maintain the optimal tone of gut barrier function and the mucosal immune system[83]. Multiple mucosal defences maintain compartmentalization of gut micro biota within the intestinal lumen, including the specialized mucus layer, antimicrobial peptides, secreted immunoglobulins and a diverse array of mucosal lymphocytes[83]. Antibiotic treatments weaken virtually all elements of this barrier, causing collapse of the mucus layer that separates epithelial cells and microbiota and reduced expression of antimicrobial peptides such as RegIIIγ, which targets Gram-positive bacteria that probably include *C. difficile*[84]. Once in contact with the epithelia, *C. difficile* toxins TcdA and TcdB inactivate small Rho-family regulatory GTPases by glycosylation, leading to disruption of essential cellular signalling pathways[85]. This step leads to weakening of the tight junctions and apoptotic death of colonocytes, which results in opening of the epithelial barrier and interaction of toxins and *C. difficile* pathogen-associated molecular patterns (PAMPs) with resident mucosal immune cells.

The mucosal immune response is the last defence before the host succumbs to systemic infections resulting from translocation of commensal microbiota across the gut wall. However, the immune response can be both protective and detrimental, which is probably dependent on multiple factors associated with the speci fic *C. difficile* strain and attributes of the individual host. For instance, the intensity of cytokine production in patients can correlate with worse outcomes. Thus, higher levels of faecal IL-8 and CXCL5, cytokines important in neutrophil recruitment, correlate with CDI severity and worse outcomes, whereas the burden of *C. difficile* bacteria does not[86].

A number of interesting insights into the immune mechanisms in CDI have emerged from studying the infection in mouse models, which highlighted the roles of *C. difficile* PAMPs, innate immune receptors, the inflammasome, as well as key cytokines and different immune cells. *C. difficile* toxins activate the inflammasome, which cleaves pro-IL-1β and pro-IL-18 to generate their mature and secreted forms[87]. The severity of toxin-induced inflammation in the colon can be reduced by blocking IL-1 or genetic deletion of different components of the inflammasome, including specific innate immune receptors in the nucleotide-binding domain leucine-rich repeat (NLR) family, the adaptor protein ASC or caspase-1 (REF. 88). However, outcomes of pure toxin-mediated colitis can differ from actual infection with *C. difficile*. In fact, CDI-associated mortality is increased in the absence of IL-1 or various inflammasome components due to increased translocation of commensals across the gut barrier[89]. Normally, the commensal microorganisms promote neutrophil recruitment by increasing IL-1 production via a positive feedback loop[89]. The massive neutrophil infiltration that is classically associated with CDI-induced pseudomembranes probably results in rapid deployment of neutrophil extracellular traps (NETs) following release of granule proteins and chromatin[90]. These NETs can patch the epithelial barrier defects and prevent microbial translocation[91]. IL-1 also enhances production of IL-23, another cytokine that protects survival of mice infected with *C. difficile*[92,93]. Importantly, IL-1 and IL-23 regulate secretion of IL-22, a cytokine that has a central role in virtually all elements of barrier maintenance, including epithelial proliferation, wound repair, as well as production

of antimicrobial peptides and mucins[94]. CDI in mice lacking IL-22 results in increased systemic infections caused by commensal γ-Proteobacteria that breach the gut barrier[95].

The need to repair the disrupted gut barrier and dampen the exuberant immune response that has been modelled in mice might be especially relevant in the context of acute pseudomembranous colitis in humans. FMT has the potential to restore the gut barrier by providing the necessary tonic signals for epithelial regeneration and production of mucins and anti microbial peptides. Indeed, it is emerging as a potentially preferable treatment approach compared with surgical options in many patients[15,16]. The need for sequential administration of multiple rounds of FMT in these situations is consistent with the time needed to repair the gut mucosa[15,16]. However, these patients can also demonstrate remarkably quick, although not necessarily sustained, favourable responsiveness to FMT in terms of haemodynamic parameters that enable prompt stabil ization of the patient[15]. This phenomenon cannot easily be explained by mechanisms of gut repair or bile-acid-mediated inhibition of *C. difficile* germination and growth. However, it is possible that certain elements of healthy, donor gut microbiota can induce rapid production of anti-inflammatory mediators that might counteract pro-inflammatory cytokines. Investigation of such possibilities are needed for patients with severe and complicated CDI being treated with FMT.

Future applications of FMT

Beyond C. difficile treatment

The increased recognition of gut microbiota as a true organ, contained within the intestine and integral to human physiology, has suggested that FMT can be applied to many diseases, including many problems pertaining to the digestive tract such as IBD and IBS[96]. This recognition has also generated speculation that FMT might be beneficial as a treatment for problems with metabolism, autoimmunity and nervous system development[96,97]. However, many challenges remain before we learn whether this approach can offer any benefit in these indications. In some respects, the issues often revolve around questions of cause versus effect. Even if dysbiotic gut microbiota are causally linked to pathophysiology of some of these problems, it is not known whether ‘normalizing’ the gut microbial community structure will result in clinical improvement once the clinical disorder is established. Possibly, certain immunological and metabolic consequences are set during a critical developmental time window in microbiota–host interaction, and cannot be reversed[98–100]. In addition, optimal protocols for gut microbiota engraftment have not yet been developed for many of these indications, and cannot be simply extrapolated from R-CDI experience with FMT. This aspect is in large part due to unique antibiotic conditioning intrinsic to current standard treatment of R-CDI[75]. Despite what might be assumed, replacing all of the gut microbiota of a recipient with that of a donor is ecologically not trivial, and requires displacement of the recipient’s autochthonous gut microbiota. Finally, the characteristics of optimal therapeutic microbiota, if such exist, might vary between different conditions. For example, gut micro biota that could be therapeutic in anorexia nervosa might be detrimental in obesity, and vice versa.

Antibiotic resistance and complications

The next major applications of FMT will probably continue to be related to complications of antibiotic treatments and emergence of increasingly more virulent, multidrug-resistant pathogens such as vancomycin-resistant enterococci (VRE), extended-spectrum β-lactamase-producing bacteria, carbepenem-resistant Enterobacteriaceae, and others. Many of these pathogens form reservoirs within the gastrointestinal tract under antibiotic pressure that is routinely applied in the context of intense medical care[101]. One example is routine antibiotic usage in patients undergoing allogeneic haematopoietic stem cell transplantation (HSCT), which leads to striking loss of microbial diversity in the gut and dominance by potential pathogens that include *Enterococcus* and γ-Proteobacteria[102]. Importantly, blooms of these specific bacteria within the intestinal tract correlate with blood-borne infections in patients undergoing HSCT, whom also undergo myeloablative conditioning with chemotherapy and radiation that are also disruptive to the gut barrier[102]. Autologous FMT using cryopreserved microbiota is already being tested in a clinical trial (NCT02269150) at the Memorial Sloan Kettering Cancer Center, USA, with the goal of testing reduction in incidence of CDI and blood-borne infections[103]. Similar issues exist within the solid organ transplant recipient population, and loss of VRE dominance has been documented following FMT performed in treatment of CDI in this setting[104].

The evolutionary forces that encourage antibiotic resistance and pathogenic potential among some commensal organisms are probably intrinsically linked. The basic symbiotic relationship based on mutual interests between the host and its gut microbiota is broken under antibiotic pressure, and commensal microorganisms seek existence outside the individual host. Many pathogens are fairly promiscuous and are able to easily move between different host species. Some are able to survive in harsh environments outside the host as spores[101,105]. Rapid evolution of antibiotic-resistant pathogens is compounded by common coexistence of both anti biotic resistance and virulence factors on mobile genetic elements that can be transferred between bacteria. FMT has the potential to restore the normal microbial gut ecology and might contribute to the range of innovative therapeutic approaches to cure infectious diseases that does not drive antibiotic resistance and might actually decrease it. We can anticipate emergence of next-generation FMT-based therapeutics that will increasingly focus on this growing challenge in medicine.

Conclusions

Rapid emergence of FMT in management of CDI has brought into focus the limitations of standard antibiotic therapies and the important roles of gut microbiota in human physiology. Mechanistic investigations of FMT have demonstrated its restorative potential for both composition and functionality of gut microbiota and suggested directions of novel therapeutic development. However, the field remains in its infancy, and interventional clinical trials coupled with mechanistic investigations will remain critical for continued progress that is needed for applications beyond *C. difficile*.

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Competing interests statement

A.K. and M.J.S. have received research grant support from Crestovo, a company working to commercialize faecal microbiota transplantation. A.K. and M.J.S. have also filed a patent application that relates to the composition and methods for transplantation of colon microbiota.

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Key points

- Faecal microbiota transplantation (FMT) involves administration of the whole microbial community from healthy donor stool into the recipient's intestinal tract to normalize or modify intestinal microbiota composition and function
- Overall suppression of microbiota and disruption of its community structure in the colon, most commonly resulting from antibiotic therapies, is the fundamental problem underlying the pathogenesis of *Clostridium difficile* infection (CDI)
- FMT results in normalization of microbial diversity and community structure in patients being treated for CDI, with high rates of clinical cure
- The restored colon microbial community could inhibit *C. difficile* by multiple mechanisms: competition for nutrients; direct suppression by antimicrobial peptides; bile-acid-mediated inhibition of spore germination and vegetative growth; and activation of immune-mediated colonization resistance

Box 1

Definition of FMT

Faecal microbiota transplantation (FMT) is a treatment that involves administration of minimally manipulated microbial community from stool of a healthy donor into the patient's intestinal tract. The notion of 'minimally manipulated' material distinguishes FMT from defined consortia of microorganisms and explicitly acknowledges the high degree of complexity and functionality of natural microbiota that might be difficult to reproduce at this stage of microbiome science. Clinically, FMT is performed with the intent of restoring normal function of the gut microbiota. Most regulatory agencies in the world that have considered this form of treatment have categorized it as a 'drug'. However, gut microbiota can be also viewed as an organ or tissue composed of complex microbial communities that have co-evolved with their human hosts. Many investigators, therefore, consider FMT a form of tissue transplantation[106]. The gut microbiota has the potential to affect many physiological functions, including energy metabolism, immunity and even neurological development. Thus, in addition to infectious risks, there are potential long-term risks for the recipient that should be considered in clinical practice of FMT. Careful donor selection, which is currently done solely through clinical evaluation of the donor rather than via metrics of gut microbiota composition, can theoretically mitigate these risks[19,107].

Box 2

Bile acid production and role

Bile acids and their conjugates are amphipathic end products of cholesterol metabolism. In addition to their role in lipid digestion and absorption, bile salts are signalling molecules that have multiple physiological effects in energy metabolism and immune responses. Primary bile acids produced in the liver are modified in the colon by indigenous gut microbiota to produce secondary bile acids. Bile acids can modulate composition of indigenous microbiota, and different bile acids can have major effects, both stimulatory and inhibitory, on the lifecycle of *C. difficile*[61].

- Bile acids in the cholic acid class generally promote *C. difficile* spore germination. The primary bile salt taurocholate is typically used in laboratory *C. difficile* growth media.
- Lithocholic acid, a secondary bile acid, is an inhibitor of *C. difficile* spore germination.
- Combinations of bile acids present at physiological concentration in healthy adult faeces (lithocholic acid and deoxycholate) are inhibitory to *C. difficile*[61].
- Combinations of bile acids found in faeces of patients with refractory R-CDI before faecal microbiota transplantation (taurocholate, cholate, chenodeoxycholic acid) are stimulatory to *C. difficile*[61].

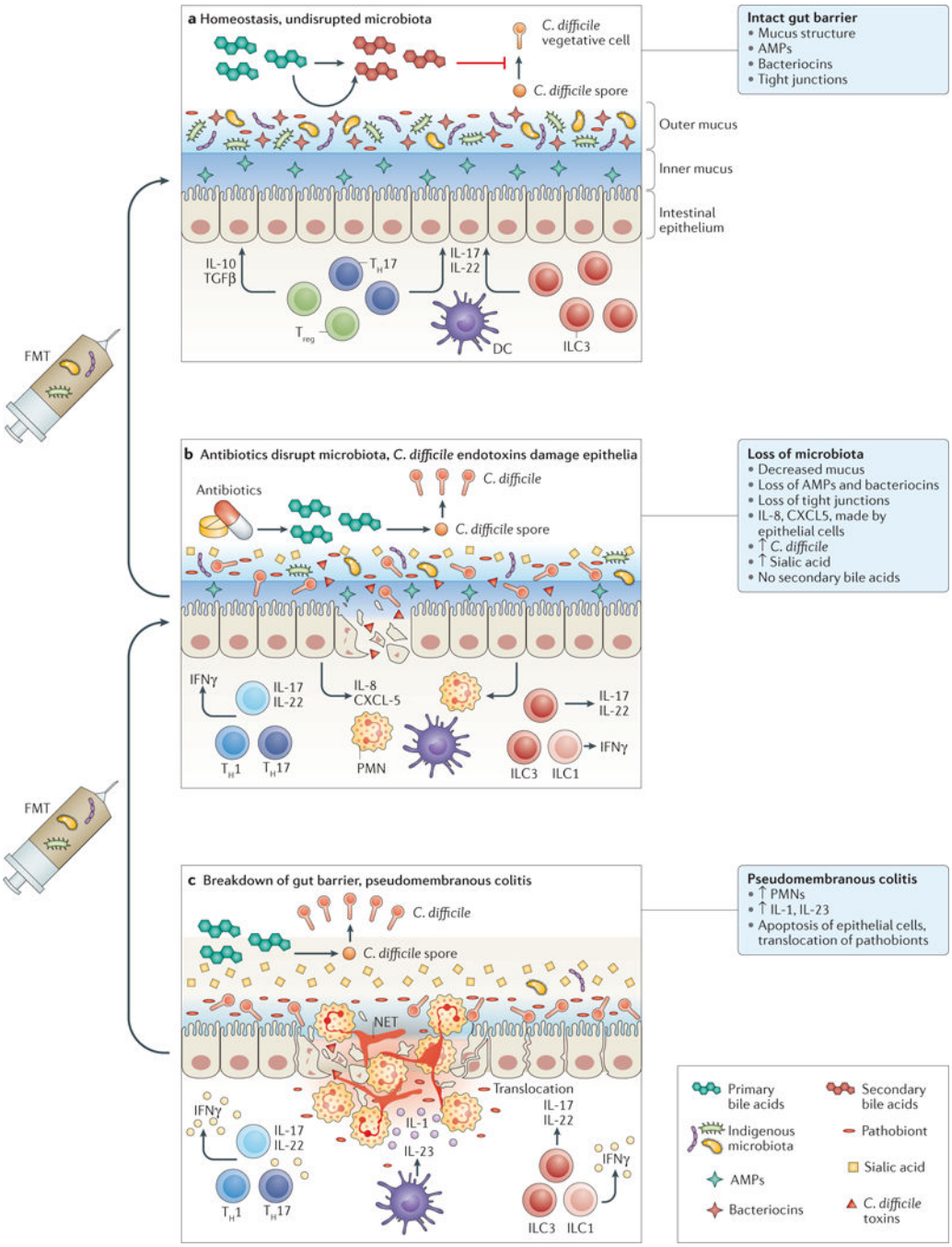

Figure 1. Loss of indigenous intestinal microbiota leads to vulnerability to *C. difficile* infection

a At homeostasis, the indigenous gut microbiota is compartmentalized within the intestinal lumen. The gut barrier is maintained by organized mucus, antimicrobial peptides (AMPs) produced by the host and the microbiota, and tight junctions between the epithelial cells. Gut microbiota provides tonic stimulation to the mucosal immune system, leading to production of different cytokines, such as transforming growth factor (TGF)β and IL-22, which help to fortify the gut barrier defences. Competition for nutrients is intense. Gut microbiota also carry out transformation of primary bile acids, which lead to a secondary bile acid

composition that is inhibitory to *C. difficile* spore germination and growth. **b** Antibiotic treatment suppresses most indigenous gut microbiota, although many drug-resistant microorganisms, which are also more likely to assume pathogenic roles themselves, remain intact. More nutrients, such as free sialic acid, become available to *C. difficile*. Primary bile acids are no longer converted to secondary bile acids, and the total bile acid composition becomes more favourable to *C. difficile* spore germination and growth. Production of mucus and AMPs is decreased. *C. difficile* enterotoxins lead to weakening of tight junctions and epithelial cell death. Neutrophils are recruited by chemokines, such as IL-8 and CXCL5. **c** Severe pseudomembranous colitis results from breakdown of gut barrier and translocation of residual microbiota. This step leads to activation of the inflammasome and production of IL-1. Massive recruitment of neutrophils occur, which form the pseudomembranes. Neutrophils release their DNA to form neutrophil extracellular traps (NETs), which form temporary patches in the broken gut barrier. T cells and innate lymphocytes produce more pro-inflammatory cytokines in response to loss of microbiota compartmentalization, which might further damage the gut epithelium and gut barriers. Faecal microbiota transplantation (FMT) restores the normal composition of gut microbiota. Severe pseudomembranous colitis typically requires multiple sequential applications of FMT. DC, dendritic cell; ILC, innate lymphoid cell; PMN, polymorphonuclear leukocyte; T_H, T helper cell; T_{reg}, regulatory T cell.