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MINIREVIEWS

Immunological mechanisms of fecal microbiota transplantation in recurrent *Clostridioides difficile* infection

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Abstract

Fecal microbiota transplantation (FMT) is a successful method for treating recurrent *Clostridioides difficile* (*C. difficile*) infection (rCDI) with around 90% efficacy. Due to the relative simplicity of this approach, it is being widely used and currently, thousands of patients have been treated with FMT worldwide. Nonetheless, the mechanisms underlying its effects are just beginning to be understood. Data indicate that FMT effectiveness is due to a combination of microbiological direct mechanisms against *C. difficile*, but also through indirect mechanisms including the production of microbiota-derived metabolites as secondary bile acids and short chain fatty acids. Moreover, the modulation of the strong inflammatory response triggered by *C. difficile* after FMT seems to rely on a pivotal role of regulatory T cells, which would be responsible for the reduction of several cells and soluble inflammatory mediators, ensuing normalization of the intestinal mucosal immune system. In this minireview, we analyze recent advances in these immunological aspects associated with the efficacy of FMT.

Key Words: Fecal microbiota transplantation; Immunity; Mechanism; Dysbiosis; Pseudomembranous colitis; *Clostridioides difficile*

Core Tip: Fecal microbiota transplantation (FMT) is an excellent treatment option of pseudomembranous colitis due to *Clostridiodes difficile* infection (CDI) because of its remarkable effectiveness. Moreover, FMT is a promising therapy for several other disorders in which dysbiosis is an important pathological factor. The mechanisms of FMT have begun to be dissected and include the restoration of the commensal microbial community structure and the modulation of several components of the immune system. This minireview focus on the FMT immune-related mechanisms for CDI.

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INTRODUCTION

The human microbiota is a complex community of microorganisms that reside on the skin and mucosal surfaces, with gut microbiota being by far the most studied microbial subcommunity[1]. Firmicutes and Bacteroidetes are the most prevalent phyla in the human gut, followed by Actinobacteria and Proteobacteria[2]. Interestingly, mammals directly or indirectly receive signals from the microbiota for adequate development and functioning throughout life[3]. These signals are important for several systems of the human body. The interaction of the microbiota with the immune system is probably the best example of how important the commensal microbiota is for the host, given that the absence of microbiota results in an immune system with fewer and less varied components, as well as delayed immune responses[3,4]. Moreover, the presence of a normal microbiota restricts the colonization of pathogens by direct and indirect mechanisms. This function of the microbiota is known as colonization resistance[5,6]. Furthermore, the alteration of intestinal microbiota composition is called dysbiosis and commonly results in disease development.

CLOSTRIDIOIDES DIFFICILE INFECTION AND INTESTINAL DYSBIOSIS CORRECTION WITH FMT

Clostridioides difficile (*C. difficile*) is a spore-forming bacillus with the capacity to retain crystal violet staining, denoting that its cell wall is rich in peptidoglycans and, therefore, becomes positive in the staining procedure created by Hans Christian Gram in 1884[7]. Although *C. difficile* could be part of the intestinal commensal microbiota, toxin-producing strains are pathogenic. Nonetheless, the ingestion of toxin-producing *C. difficile* does not necessarily result in disease development because the microbiota is able to avoid colonization and overgrowth of this pathobiont[8]. However, *C. difficile* infection (CDI) is well known to occur due to a combination of two factors: (1) Ingestion of the bacillus spores during hospitalization, where the circulation of strains capable of expressing toxins A, B, and C - which damage the intestinal epithelium - is more common; and (2) Receiving or having recently received broad-spectrum antibiotic therapy, which will cause intestinal dysbiosis[8,9]. Thus, antibiotic exposure followed by acute episodes of diarrhea is the main clinical indicator of CDI. The detection of toxins associated with colonoscopic and/or histopathologic findings will confirm the diagnosis of pseudomembranous colitis[10]. Elderly persons are more affected by the disease; however, CDI is becoming more frequent in younger populations and with no association with previous hospitalizations[8,11]. The emergence of hypervirulent and antibiotic-resistant *C. difficile* strains contributed to the burden of worldwide cases of antibiotic-associated diarrhea and pseudomembranous colitis[8,10]. In fact, CDI may range from mild or self-limiting diarrhea to severe cases and the development of sequelae, including toxic megacolon and fulminant colitis. CDI is commonly treated by antibiotics (Metronidazole, Vancomycin, and Fidaxomicin) with efficacy rates ranging from 76% (Metronidazole) to as high as 97% (Vancomycin and Fidaxomicin)[12,13]. However, as with many other broad-spectrum antibiotics, *C. difficile* can also develop resistance mechanisms to these and other antibiotics[14]. Furthermore, antibiotic therapy, which treats CDI, will enhance dysbiosis and will predispose the patient to CDI relapse[15]. In fact, it is well known that 20%-30% of antibiotic-treated CDI cases subsequently develop recurrent episodes of the infection (rCDI)[16-18].

FMT is primarily indicated for treating pseudomembranous colitis due to rCDI[19,20]. The use of FMT for rCDI is based on several studies reporting the effectiveness of FMT, supporting it as the most effective treatment for this disease. In a systematic review on FMT effectiveness against rCDI that included 45 studies (36 cohort studies and nine randomized clinical trials), it was shown that FMT has

91% effectiveness after eight weeks of repeated treatment - far superior to the use of antibiotics[21]. According to the United States Food and Drug Administration, FMT may be performed after two failed courses of antibiotics[22]. The fecal material for FMT may be obtained from a relative or unrelated donor and administered using a nasogastric or nasoduodenal tube or by colonoscopy[19,20,23,24]. More recently, successful FMT treatments using lyophilized solutions and capsules have been reported[25-27]. Commonly, the administration of one or two courses of FMT results in clinical remission as early as one day after the first FMT[23,24,28]. Its effects are based mainly on the restoration of eubiosis[29]. This implies that FMT effectiveness relies on microbiologic mechanisms, or in other words, the restoration of colonization-resistance-related mechanisms[30-32]. However, indirect mechanisms of colonization resistance include the crosstalk with different components of the immune system, which will be important for both maintaining the integrity of the intestinal mucosa or restoring that integrity if the disease is already present, as is the case of pseudomembranous colitis due to CDI[9,33].

Notably, FMT restores the capacity of the microbial community to convert primary bile acids (BAs) into secondary BAs, such as deoxycholic acid and ursodeoxycholic acid, which can inhibit *C. difficile* germination and epithelial apoptosis[34]. Although not directly shown in FMT, the optimal biotransformation of BAs by microbiota also modulates the repertoire and functions of colonic RORγt+ T regulatory (Treg) cells, contributing to intestinal homeostasis[35]. Moreover, higher levels of primary BAs in the stool, such as taurocholic acid - which can promote the spore germination of *C. difficile* - have been reported in rCDI patients compared to healthy individuals as well as compared to patients experiencing their first episode of CDI[36]. This is compatible with the bile salt hydrolase (*BSH*) gene abundance reduction - which metabolizes BAs - in rCDI patients compared to healthy and first episode CDI individuals[24]. Furthermore, BSH functional activity is rapidly restored in rCDI patients after FMT [23]. Taken together, these data indicate that gut microbial BAs metabolism is one of the molecular mechanisms of FMT to successfully treat rCDI.

Similarly, the recovery of microbiota functions after FMT is linked to the repopulation of short-chain fatty acids (SCFAs) producer bacteria - mainly members of the Clostridiales clade that include several butyrate producers[37]. SCFAs are known to serve as the main source of energy for colonocytes but also play a role in homeostasis maintenance, inducing the differentiation into effector and Treg cells in the intestinal lamina propria (LP)[38]. BAs and SCFAs are bacterial metabolites with pleiotropic effects on the immune system but, acting together, they may play a crucial role in reducing the inflammation in the intestine after FMT[35].

One important aspect of FMT refers to means of improving it by using simpler preparations that could offer more standardized formulations, being more patient-friendly, and avoiding any type of potential risks by not using an undefined combination of living microorganisms, as is the case with FMT. In this regard, Feuerstadt *et al*[39] have recently reported the use of oral capsules composed of live purified Firmicute bacterial spores in a phase 3 clinical trial of patients with rCDI. Of the 89 patients treated with this formulation and followed for eight weeks, they observed recurrence in 11 patients (12%) compared to 37 patients (40% of recurrence) in the placebo group[39].

On the other hand, Zhang *et al*[40] proposed to submit the fecal material of standard FMT to a combinatorial method of filtration and centrifugation to offer a safer, more precise and quality-controllable microbiome transplant. The authors called this material "washed microbiota transplantation" (WMT) and provided evidence of reduced levels of pro-inflammatory molecules such as leukotriene B4, corticosterone, and prostaglandin G2 in mice which were intraperitoneally injected with WMT. Despite that washed microbiota has been used successfully to treat ulcerative colitis and Crohn's disease since 2014, it has not been evaluated in the context of rCDI[40].

Interestingly, Ott *et al*[41] showed that a single administration of sterile fecal filtrate (FFT), which contains bacterial components, bacteriophages, and bacteriocins but not whole bacterial cells, was able to eliminate symptoms and avoided the recurrence of CDI in 5 patients. This finding could overturn the necessity of living bacteria and successful engraftment of donor microbiota to reach the protective effect of FMT in rCDI patients. A possible explanation for this result could be that bacteria cell wall components and DNA fragments, which remain after filtration, stimulate the host's innate immune responses, with subsequent reprogramming of the mucosal immune mechanisms against the pathogen while promoting the restoration of homeostasis. The authors also proposed an additional explanation in which the massive transfer of bacteriophages from the donor to the host would be able to correct dysbiosis in rCDI patients. Although the study does not assess BAs or SCFAs content in the FFT, it is reasonable to consider that these metabolites could also participate in the effects reached by FFT since they are expected to persist after the filtration process. The absence of potential bacterial pathogens in the transplanted material - as is the case when using FFT - could represent an important advantage for the use of FFT in immunodeficient patients instead of living bacteria. In addition, FFT could also be better standardized. Therefore, FFT needs to be explored in detail in a larger group of patients and compared to FMT. Figure 1 summarizes these FMT variations and their key features.

Taking together all these studies, one may conclude that immune pathways activated during the response to *C. difficile* are important not only to identify the mechanisms that effectively contribute to its elimination but also to determine which immune components are activated or respond to FMT.

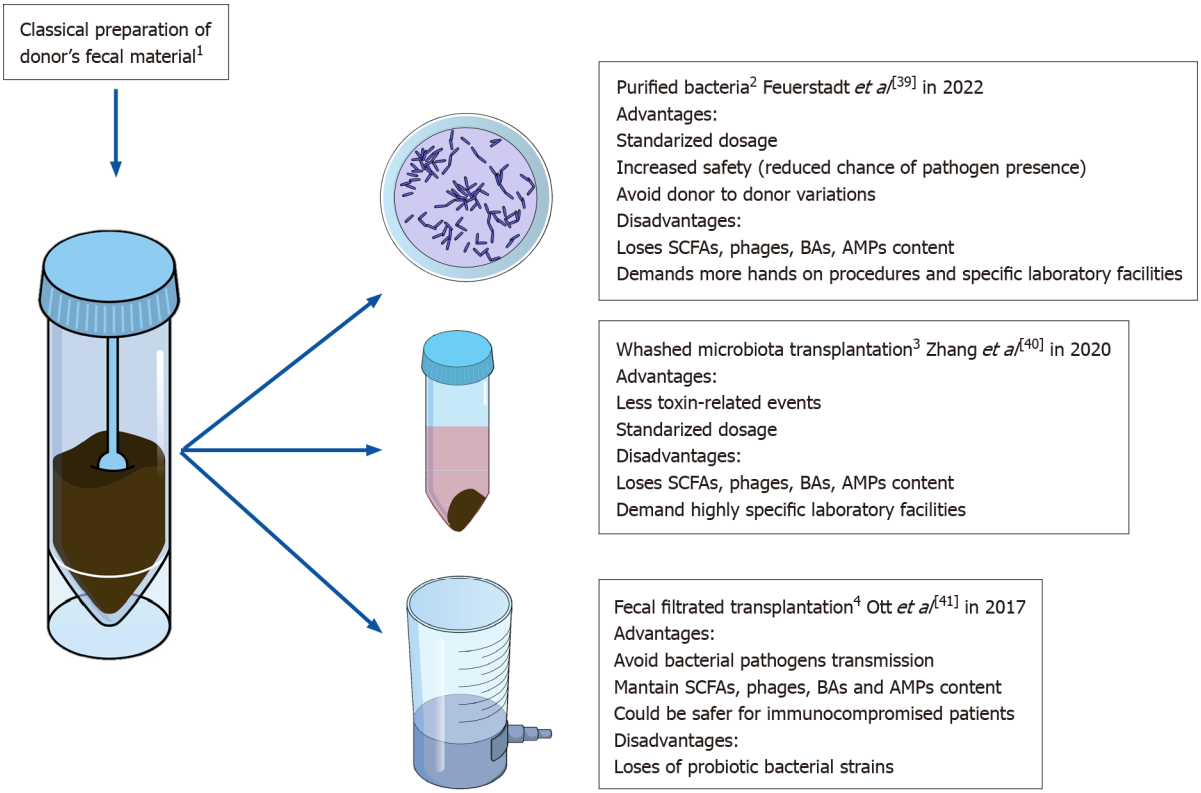

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Figure 1 Advantages and disadvantages of non-classical preparations methods of donor's fecal material prior to fecal microbiota transplantation. [1]Classical preparation consist in dissolve donor's fecal material by blending with saline water and filter out residual solid feces through gauze or fabric. [2]Isolation of different bacteria strains directly from donor's fecal material. [3]Basically, this method consists in consecutively centrifugation of microbiota from donors to remove the supernadants. [4]Uses filtration systens to retain debris and bacterial load from the donor's fecal material. SCFAs: Short-chain fatty acids; AMPs: Antimicrobial peptides; BAs: Bile acids.

ESSENTIAL CONCEPTS OF THE IMMUNE RESPONSE DURING CDI

As there are recent and excellent reviews on the immune response to *C. difficile*[10,42,43], in this section, we present the main characteristics of this host-pathogen interaction.

The immune response to *C. difficile* is characterized by the development of an inflammatory reaction with Th1 and Th17 components. This response starts with bacterial sensing by epithelial cells and the release of interleukin (IL)-1 and IL-8 with high capacity to attract neutrophils[44,45]. Type-1 innate lymphoid cells (ILC-1) also participate in the response by secreting interferon-γ (IFN-γ)[46]. Antigen-presenting cells (APCs), including macrophages and dendritic cells (DCs), are important to capture and process *C. difficile* antigens, migrate to draining lymph nodes, and activate specific T cells[47]. Under these circumstances, Th1 cells are generated, but the secretion of IL-6 and IL-23 provide sufficient stimuli for the expansion of Th17 cells[48]. While these aspects of the immune response could be pivotal for appropriate enhancement of several bacteria-killing mechanisms by innate cells, it is already known that an exacerbated immune response signifies the development of pseudomembranous colitis, which is the histopathological lesion caused by the inflammatory response taking place in the colon[49]. To avoid the development of an immunopathological response, two important branches of immunity are required. First, the activation of Treg cells with the secretion of immune regulatory cytokines IL-10 and transforming growth factor-β (TGF-β)[50]. The source of these regulatory cytokines may also be enriched from other cell subsets such as intestinal epithelial cells, for example, which have the ability to secrete relevant quantities of TGF-β[50,51]. Secondly, recent studies have indicated the importance of active Th2 components present in patients with CDI who do not develop histopathological lesions but, instead, resolve the infection. These elements include mainly ILC-2 and eosinophils as the main cell populations[52,53], as well as type 2 cytokines, including IL-4, IL-5, IL-13, IL-25, and IL-33[54-56].

While the participation of Th1, Th17, and Treg cells during the response to bacteria with the characteristics of *C. difficile* is easy to understand, the type 2 component - which appears to have remarkable importance for the host to avoid an overreacting inflammatory response - is unexpected. Therefore, the antigenic components responsible for the activation of ILC-2 and eosinophils during CDI are new essential factors to be identified to better understand the effective immune response against *C. difficile*.

Table 1 Main clinical and experimental studies about the immunological mechanisms associated to fecal microbiota transplantation efficacy

Ref.	Model / clinical study	Main findings
Ekmekciu *et al*[57], 2017	Mice treated with antibiotic cocktail followed by FMT	Recovery of INF-γ, IL-17, IL-22 and IL-10 producer CD4+ T cells in intestinal LP. FMT failed to recover CD8+ T and B cells in LP after antibiotic exposure
Burrello *et al* [58], 2018	Mice treated with DSS followed by oral gavage of mucus and feces from healthy mice	Treatment increased Camp, S100A8, Muc1 and Muc4, reduced MHC-II+ cells and normalized populations of ILC-2, ILC-3, F4/80+ macrophages and CD11b+ Ly6G+ neutrophils
	In vitro stimulation of LPMCs stimulated with FMT or DSS-derived microbiota	FMT reduced IL-1β, TNF-α and IFN-γ and increased IL-10
	IL-10 receptor blockade in DSS mice prior of FMT treatment	Blockade of IL-10 resulted in reduction in colon length, increased weight loss and expression of *IL-1β, TNF and IFNγ* genes
Littmann *et al*[59], 2021	Use of different KO mice to evaluate B and T (CD8+, Th1, Th17 and Treg) cells	Treg cells play a pivotal role for FMT to achieve the effects against CDI
Monaghan *et al*[60], 2021	Multiomic analysis of fecal, sera and PBMC samples of patients with severe (n = 3) or fulminant (n = 1) CDI treated with FMT in 6 occasions plus fidaxomicin (severe cases) or vancomycin and metronidazole (fulminant case)	One patient (severe CDI) did not respond to treatment. The fulminant case responded after FMT in 10 occasions plus antibiotics. 78 features were identified differentiating responders to the non-responder. Non responsiveness was associated to higher levels of MMP-2, TWEAK, IL-26, sTNF-R1, sTNF-R2, effector memory CD8 T cells and circulation of senescent T cells; and lower TCR diversity repertoire, B cell and regulatory B cell frequencies

IL: Interleukin; FMT: Fecal microbiota transplantation; INF: Interferon; LP: Lamina propria; DSS: Dextran sodium sulfate; LPMCs: Lamina propria mononuclear cells; Muc: Mucin; TNF: Tumor necrosis factor; Treg: T regulatory; KO: Knockout; CDI: *Clostridioides difficile* infection; PBMC: Peripheral blood mononuclear cell; ILC: Innate lymphoid cells.

IMMUNOLOGICAL EFFECTS ASSOCIATED WITH FMT EFFICACY TO TREAT RCDI

One important attempt to gain knowledge to elucidate the immunological events elicited by FMT to successfully treat rCDI was made by Ekmekciu *et al*[57]. In this article, the investigators evaluated the effects on the immune system of mice treated for eight weeks with a cocktail of five antibiotics followed by FMT to resolve the dysbiosis caused by the antibiotic exposure. After successfully showing the depletion of the intestinal microbiota and reduction of proliferating cells in the intestines, as well as the restoration of both parameters soon after FMT, the authors evaluated different populations of the immune system. The investigators found that CD4+ T lymphocyte frequencies decreased after antibiotic treatment in the gut LP and mesenteric lymph nodes and presented recovery from the seventh day after FMT. Paradoxically, the absolute number of CD4+ T lymphocytes increased in the spleen and did not return to normal levels 28 d after FMT. CD8+ T cells showed the same profile as CD4+ T cells in LP, mesenteric lymph nodes, and the spleen, but not in the colon. The number of colonic CD8+ T cells presented a huge reduction after the antibiotic treatment, and FMT failed to induce the recovery of these cells. In parallel, B cells presented equivalent alterations to those of T cells in the gut (including an important reduction in the colon with no recovery after FMT), mesenteric lymph nodes, and the spleen, yet only in absolute cell numbers and not in cell frequencies[57].

The microbiota depletion due to the antibiotic treatment also resulted in a reduction of T cells with memory/effector phenotype ($CD44^{hi}$), Tregs, and co-stimulatory molecules in DCs, with the restoration of all these parameters after FMT. The authors also found that IFN-γ, IL-17, IL-22, and IL-10-producing T cells decreased with antibiotic treatment but were restored with FMT[57].

Subsequently, using the classical dextran sodium sulfate (DSS) colitis model, Burrello *et al*[58] provided a more profound and dynamic analysis of the effects of FMT in resolving intestinal inflammation. They used the $CXCR6^{egfp}$ reporter mice, in which T cells may be tracked, including the invariant natural killer T cell population. The investigators treated mice with DSS for seven days and, after a two-day recovery period, the mice received FMT on three consecutive days. The mice received a preparation of intestinal mucus on the first day and feces from healthy donor mice on the second and third days. Evaluations were performed one and five days after the last FMT. They found that FMT reduced the production of IL-1β and increased the production of antimicrobial peptides (Camp and S100A8) and mucins (Muc1 and Muc4), all in the colonic epithelium. In parallel, they observed effects on the innate and adaptive immune systems in DSS colitic mice treated with FMT. In the innate immune system, DSS inflammation induced the expansion of ILC-2 and ILC-3, F4/80+ macrophages, and CD11β+ Ly6G+ neutrophils, followed by a reduction of these populations after FMT. Furthermore, FMT strongly reduced MHC-II+ cells, indicating that the bacteriotherapy also affected APCs. These observations were correlated with the evaluation of LP mononuclear cells (LPMCs) stimulated with FMT or DSS-derived microbiota, *in vitro*. They found that innate and adaptive LPMCs stimulated with FMT-derived microbiota produced less IL-1β, tumor necrosis factor-α, and IFN-γ pro-inflammatory cytokines while increasing the production of IL-10. Moreover, the investigators went further, attempting to determine

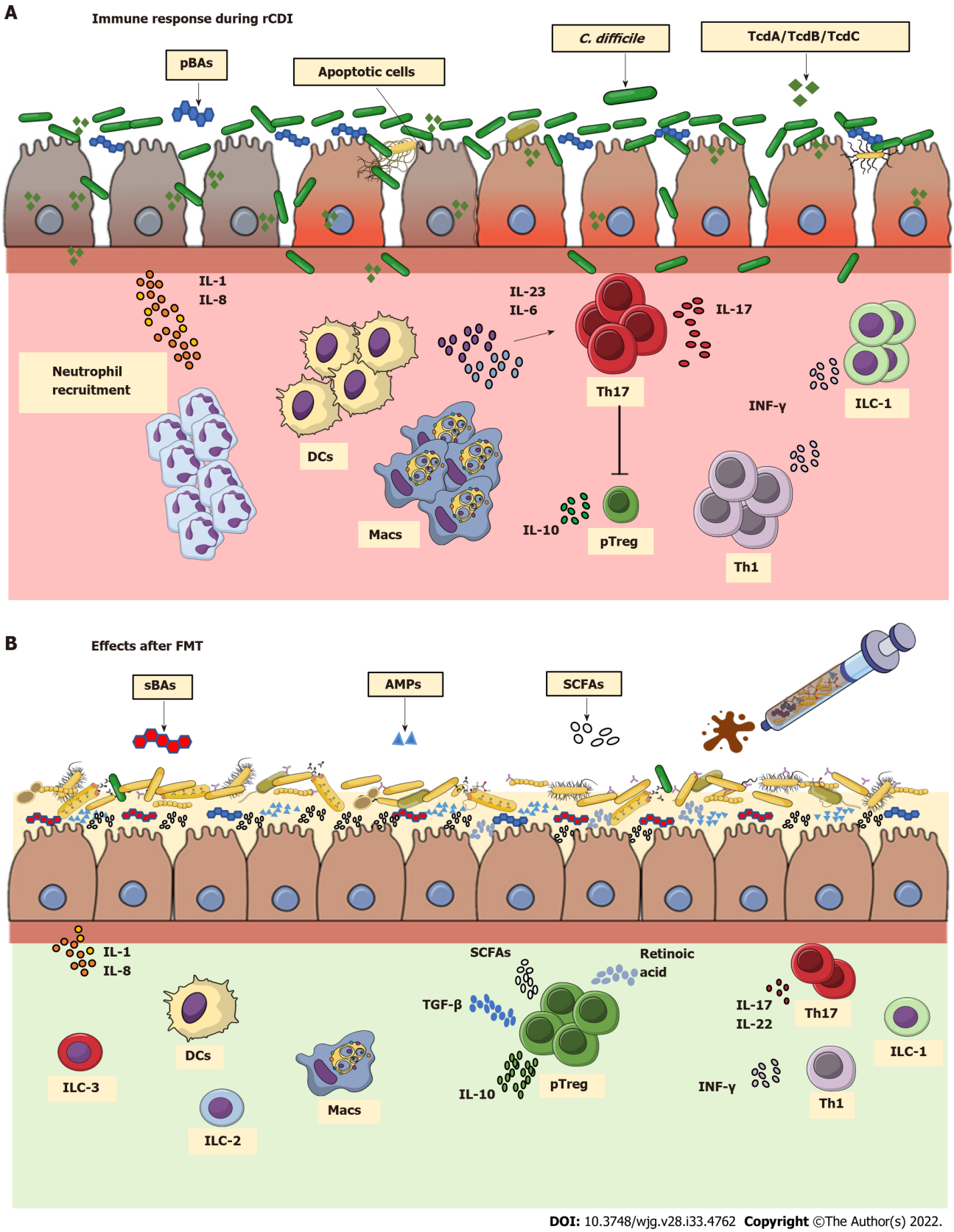

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Figure 2 The immune response during recurrent *Clostridioides difficile* infection and after fecal microbiota transplantation treatment. A: During recurrent *Clostridioides difficile* (*C. difficile*) infection, the depletion of commensal microbiota results in higher levels of primary bile acids (BAs). These molecules are known to trigger the *C. difficile* vegetative state and its expansion, resulting in production of toxin (Tcd) A, TcdB, and TcdC, which cause apoptosis of enterocytes and release of interleukin (IL)-1 and IL-8 in the lamina propria (LP). Consequently, there is extensive recruitment of neutrophils dendritic cells, and macrophages and type 1 innate lymphocytes (ILC-1) in an attempt to cope with the infection. These cells produce pro-inflammatory cytokines including IL-6, IL-23, interferon (IFN)-γ, which induce Th17 and Th1 differentiation. This inflammatory state dominated by IL-17 and IFN-γ promotes tissue damage, which could spread along the intestines, and is accompanied with absence of innate ILC-2 and reduction of peripheral T regulatory (Treg) cells; B: The therapeutic effects of FMT involve

the reestablishment of a wide variety of commensal microorganisms that directly and indirectly antagonize *C. diffcile*. Commensal strains that produce secondary Bas and short chain fatty acids are re-established, as well as the production of antimicrobial peptides by epithelial cells together with the reconstitution of the barrier integrity. These effects allow to reduce the activation of innate immunocytes, the expansion of Treg cells which produce IL-10, and subsequent normalization of Th1 and Th17 cell frequencies in the LP. IL: Interleukin; FMT: Fecal microbiota transplantation; TNF: Tumor necrosis factor; INF: Interferon; SCFAs: Short-chain fatty acids; AMPs: Antimicrobial peptides; TGF: Transforming growth factor; pBAs: Primary bile acids; sBAs: Secondary bile acids; DCs: Dendritic cell; ILC: Innate lymphoid cells; rCDI: Recurrent *Clostridioides difficile* infection; Treg: T regulatory; Macs: Macrophages.

the importance of IL-10 secretion for the beneficial effects of FMT to treat DSS colitis. Using a concomitant administration of IL-10 blockade in DSS mice during the FMT treatment, the authors demonstrated the pivotal importance of this regulatory cytokine for inflammation resolution, including the normalization of the histological score and intestinal weight[58].

More recently, Littmann *et al*[59] were able to confirm the importance of IL-10-producing cells for the host to be able to respond effectively to FMT. Their 2021 report started by considering that if the host immune system is not important for FMT to resolve the intestinal inflammation during CDI, then immune-deficient mice should be equally effective as wild-type animals to respond to FMT. To test their hypothesis, they used *Rag1* double knockout animals (*Rag1*$^{-/-}$), which lack T and B cells, and compared CDI course before and after FMT. They observed that CDI persisted in *Rag1*$^{-/-}$ animals but not in wild-type (WT) littermates nor *Rag1*^{HET} controls. Importantly, they confirmed this observation by excluding the possibility that the microbiota composition of *Rag1*$^{-/-}$, which is known to be different from the microbiota of *Rag1*^{HET} mice, was indeed the reason for the differential responses to FMT. They were able to do so by performing three additional control experiments. First, they analyzed and compared the microbiota composition of *Rag1*$^{-/-}$, *Rag1*^{HET}, and WT mice. Second, they transferred WT or *Rag1*$^{-/-}$ microbiota to antibiotic-treated *Rag1*$^{-/-}$ and *Rag1*^{HET} mice. Third, they employed germ-free C57BL/6 mice, which were cohoused with *Rag1*$^{-/-}$ or *Rag1*^{HET} mice. These mice were treated with antibiotics, infected with *C. difficile*, and then treated with FMT. All these experiments confirmed that the difference in the responses to FMT in *Rag1-/-* or *Rag1*^{HET} depended on an adaptive immune cell population and not on differences in microbiome composition prior to FMT. Next, the investigators focused on determining which adaptive immune cell population is important for FMT efficacy. By using specific knock-out animals, they excluded the importance of B cells, CD8+ T cells, Th17, and Th1 CD4+ cells. In contrast, the transient specific ablation of Treg cells using the diphtheria toxin Foxp3-DTR mice demonstrated that Treg cells are pivotal to observing the effects of FMT against CDI. Moreover, Littman *et al*[59] also showed that FMT engraftment is different depending on the immune activation status of the host, as well as that the microbiota post-FMT is metabolically distinct depending on the functionality of the host immune system.

Finally, Monaghan *et al*[60] performed a systems biology-based study to interrogate the interaction among microbiome-metabolome-immune system in the context of FMT applied to patients with severe or fulminant CDI. They studied four patients unresponsive to antibiotic therapy and treated with sequential FMT. Three patients were responders against one non-responder. The evaluations included microbiome and associated metabolome profile in fecal samples as well as the evaluation of blood samples to access the epigenomic, metabolomic, glycomic, immune proteomic, immunophenotyping, functional immune assays, and the T cell receptor repertoire. Although the small sample size did not allow the authors to draw clear conclusions, they suggest that immunosenescent signals could be associated with non-responsiveness to FMT, since they found strong correlations between peripheral senescent T cells and host factors including butyrate, serum hydroxybutyrate, fecal urso- iso- and hyodeoxycholic acids, serum immunoglobulin G Fc N-glycopeptides, and microbial taxa including *Pseudomonas* at the genus level[60]. The main findings of the studies recently discussed are listed in Table 1.

In summary, the studies described here indicate that FMT effectiveness rely not only on the capacity of donor eubiotic microbiota to be able to expulse *C. difficile*, but also to produce key metabolic products as secondary BAs and SCFAs. These metabolites, together with the displacement of the pathogen which represents less injury and consequently reduction of pathogen-derived antigens for innate and adaptive immunity activation, allow Treg cells to expand and increase the production of IL-10. This regulatory activity becomes pivotal for different types of immune cell populations and their produced cytokines to return to normal levels dampening inflammation, as shown in Figure 2.

CONCLUSION

The findings discussed here provide a new perspective on the therapeutic effects of FMT to restore eubiosis. This ability refers, in our opinion, two key aspects: (1) The material to be transplanted must contain the appropriate elements to displace the pathogen and modulate the immune system of the patient effectively; and (2) The status of the immune system of the patient is decisive at the moment of receiving the transplant, which means that the patient's immune system must be able to respond

adequately to the FMT stimuli.

FMT is an effective option for the resolution of rCDI and is being used around the world increasingly. Moreover, recent studies show its efficacy in treating the first episode of CDI as well as its repeated use can treat severe and fulminant CDI forms[60]. Although the concept of the method is simple, it is a labor-intensive procedure and requires the acceptance of the patient to be treated with this kind of transplant, even if FMT-derived capsules are used. Nonetheless, as FMT is increasingly showing its benefits in a variety of clinical situations (*e.g.*, autism spectrum disorders, type 2 diabetes, and, of course, different types of inflammatory bowel diseases), this should guarantee not only the continuing use of FMT but also the advancement of basic research seeking to identify the molecular microbiological components that are pivotal for FMT efficacy. In addition, the new evidence discussed here shows the importance of disclosing in detail the immunological pathways that must be activated/deactivated during the FMT process. At the same time, it is necessary to consider that several of these observations came from animal studies, and where FMT was used to treat colitis induced by DSS or antibiotic cocktails but not by *C. difficile* infection. Nonetheless, all these efforts should lead to the identification of molecular factors that may become candidates for the development of new and more conventional therapeutic products that could replace FMT in the future or improve its results.

FOOTNOTES

Author contributions: Soveral LF, Korczaguin GG, and Schmidt PS collected the literature and wrote the first draft, conceptualized the table and figures, and contributed equally to this work; Nunes IS and Fernandes C corrected the first draft; Zárate-Bladés CR conceptualized the structure of the text and critically revised the manuscript for important intellectual content; and all authors read and approved the final version of the manuscript.

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