Single Delivery of High-Diversity Fecal Microbiota Preparation by Colonoscopy Is Safe and Effective in Increasing Microbial Diversity in Active Ulcerative Colitis

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Background: Recent trials suggest fecal microbiota transplantation (FMT) with repeated enemas and high-diversity FMT donors is a promising treatment to induce remission in ulcerative colitis.

Methods: We designed a prospective, open-label pilot study to assess the safety, clinical efficacy, and microbial engraftment of single FMT delivery by colonoscopy for active ulcerative colitis using a 2-donor fecal microbiota preparation (FMP). Safety and clinical endpoints of response, remission, and mucosal healing at week 4 were assessed. Fecal DNA and rectal biopsies were used to characterize the microbiome and mucosal $CD4^+$ T cells, respectively, before and after FMT.

Results: Of the 20 patients enrolled in this study, 7 patients (35%) achieved a clinical response by week 4. Three patients (15%) were in remission at week 4 and 2 of these patients (10%) achieved mucosal healing. Three patients (15%) required escalation of care. No serious adverse events were observed. Microbiome analysis revealed that restricted diversity of recipients pre-FMT was significantly increased by high-diversity 2-donor FMP. The microbiome of recipients post-transplant was more similar to the donor FMP than the pretransplant recipient sample in both responders and nonresponders. Notably, donor composition correlated with clinical response. Mucosal $CD4^+$ T-cell analysis revealed a reduction in both Th1 and regulatory T-cells post-FMT.

Conclusions: High-diversity, 2-donor FMP delivery by colonoscopy seems safe and effective in increasing fecal microbial diversity in patients with active ulcerative colitis. Donor composition correlated with clinical response and further characterization of immunological parameters may provide insight into factors influencing clinical outcome.

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Key Words: microbiome, fecal microbiota transplantation, ulcerative colitis

Fecal microbiota transplantation (FMT) has emerged as an effective therapy for recurrent *Clostridium difficile* infection, and increased microbial diversity is a characteristic feature of a successful responder[1]; however, the efficacy of FMT for inflammatory bowel disease (IBD) remains unclear. Although the etiopathogenesis of IBD is thought to be multifactorial, alterations in the intestinal microbiome are characteristic of both Crohn's disease and ulcerative colitis (UC).[2] Given that extensive characterization in

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animal models supports a role for the microbiome in driving aberrant inflammatory disease in a genetically susceptible host, several studies have recently sought to evaluate the role for FMT in the treatment of IBD.

Three randomized controlled trials of FMT for treatment of active UC were recently reported with mixed clinical efficacy.[3–5] The TURN trial (N = 50) which delivered FMT through nasoduodenal tube at week 0 and week 3 showed no significant change in clinical remission between subjects who received donor or autologous stool.[3] By contrast, a 6-weekly fecal enema–based FMT study (N = 75)[4] and a multidonor colonoscopic infusion followed by 5 d/wk enema for 8 weeks (N = 85)[5] showed significant improvement in clinical remission. Despite differences in primary clinical endpoints, all studies showed effective engraftment of a donor microbiota with increased diversity. Although differences in diversity between responders and nonresponders did not meet significance, donor microbial composition correlated with treatment success.[4,5] Differences in patient population, dosing regimen, and delivery modalities may also account for discordant results between these studies. Repeated delivery of fecal enema may provide more effective delivery than nasoduodenal delivery or single colonoscopic delivery, which was not clinically effective in smaller studies.[6] The rational utilization of engraftment metrics, donor composition, and delivery modality remain critical outstanding questions in FMT design.

To help address these questions, we performed a single-center, prospective, open-label pilot study to evaluate the safety and efficacy of 2-donor fecal microbiota preparation (FMP) delivery by colonoscopy. By combining donors for the FMP, this strategy allowed us to evaluate the efficacy of high-diversity FMP and differences in donor microbial composition in relation to clinical response. Immune cell profiling was performed on mucosal biopsies before and after FMT to assess the impact on mucosal T-cell immunity.

MATERIALS AND METHODS

Patient Selection

This study was registered with ClinicalTrials.gov (NCT02516384, IND 15988). Potential FMT subjects underwent interview and physical examination to determine eligibility. Criteria for inclusion were age ≥ 18 years old at the time of enrollment and biopsy-proven UC with active disease as defined by Mayo score ≥3 and an endoscopic subscore ≥1. Only patients on stable doses of mesalamine, immunomodulator, biologics, and prednisone before screening were included. All patients on prednisone were taking 5 mg or less. Patients were excluded if they met any of the following criteria: biopsy-proven Crohn's disease or indeterminate colitis, acute abdomen or other clinical emergencies requiring emergent management, primary sclerosing cholangitis, pregnancy, concurrent *C. difficile* infection or other infection, history of FMT, antibiotic use within the previous 3 months, other causes of diarrhea, including but not limited to tube feeds and medications (i.e., kayaxelate, metformin, lactulose, laxatives, magnesium), major congenital defects, recent malignancy in the last 5 years excluding nonmelanoma skin cancers, anaphylactic reaction to food, or any other condition that in the investigators' opinion would jeopardize the safety or rights of the participant, would make it unlikely for the participant to complete the study.

Eligible patients underwent serological testing for HIV type 1 and 2 antibody (Ab), Hepatitis A total Ab, Hepatitis B surface antigen (Ag), Hepatitis B surface Ab, Hepatitis B core Ab (IgM and IgG), Hepatitis C Ab, CMV IgM, and RPR. They also underwent stool testing with bacterial culture for enteric pathogens (*E. coli, Salmonella, Shigella, Yersinia, Campylobacter*), ova and parasites; *C. difficile* toxin by PCR; fecal *Giardia*, *Cryptosporidium*, and H. pylori antigen; and Norovirus and Rotavirus through EIA. Women of childbearing potential had a urine pregnancy test on the day of the FMT procedure to ensure eligibility, as well as serum beta-HCG testing at each follow-up visit.

Donor Fecal Microbiota Preparation

Two-donor FMPs were provided from rigorously screened healthy donors from a universal stool bank (OpenBiome).[7] Sixty milliliters of material from each of 2 donors (total of 120 mL) was thawed, pooled, and homogenized immediately before colonoscopic delivery to the ileum and right colon. Two-donor FMPs were prepared from 4 unique donors (using a 4 choose 2 factorial design with all pairwise combinations) to generate six 2-donor FMPs each of which was used at least 3 times.

Fecal Microbiota Transplantation

All patients meeting entry criteria underwent standard polyethylene glycol-based colonoscopy bowel preparation and magnesium citrate on the day before colonoscopy. No antibiotic pretreatment was administered before FMT. Colonoscopy was performed to the terminal ileum. FMP was delivered in the terminal ileum and right colon in equal proportions. Before administration, 2 mucosal biopsies were obtained from the rectum for cellular analysis. After colonoscopy, patients were administered 4 mg of loperamide to assist with FMT retention.

Outcome Measures

Patients were followed post-FMT by medical interview and physical examination at 2, 4, and 12 weeks. Patients were maintained on stable doses of UC specific medications (i.e., corticosteroids, mesalamines, immunomodulators, and biologics) throughout this period. At each visit, patients underwent medical interview and physical examination to assess for UC activity using partial Mayo score as well as adverse reactions. In addition, patients received follow-up phone calls post-FMT at 24 hours, 1 week, and 6 weeks. Patients were instructed to notify the treating physician at any time post-FMT if they were to develop any infectious symptoms or new medical conditions. At screening (pre-FMT) and at week 2 and 4 post-FMT, fecal samples for microbiome and stool studies were collected. At week

TABLE 1. Baseline Characteristics of Recipients Before FMT

Baseline Characteristics		Range
Age (Mean, SD)	38.4 (12.6)	23–71
Sex, N (%)		
Male	12 (60)	
Female	8 (40)	
Extent of disease, N (%)		
Proctitis	1 (5)	
Proctosigmoiditis	3 (15)	
Left sided colitis	7 (35)	
Pancolitis	9 (45)	
UC Medications, N (%)		
Corticosteroids	6 (30)	
Mesalamine	11 (56)	
Anti-tumor necrosis factor α	1 (5)	
Vedolizumab	3 (15)	
Thiopurines	3 (15)	
None	2 (10)	
Biologic naive	10 (50)	
Laboratories (Mean, SD)		
WBC, $\times 10^3/\mu L$	7.4 (1.9)	4.4–10.4
Hemoglobin, g/dL	13.1 (1.3)	10.3–15.2
ESR	22.6 (19.8)	4.0–72.0
CRP, mg/L	0.9 (1.5)	0.0–6.8
Scores (Mean, SD)		
Pre-FMT total Mayo score	8.1 (2.4)	3.0–11.0
Pre-FMT endoscopic Mayo score	2.4 (0.8)	1.0–3.0

4 post-FMT, flexible sigmoidoscopy was performed to determine the endoscopic subscore and total Mayo score as well as obtain mucosal biopsies of rectal mucosa for cellular analysis.

The primary endpoint outcome of safety was evaluated by adverse events assessment within 24 hours of FMT and then at weeks 1, 2, 4, 6, and 12. Information regarding adverse events was obtained using open-ended questions along with specific questions soliciting major and minor adverse events including fevers, chills, fatigue, malaise, anorexia, abdominal pain, constipation, diarrhea, nausea, and vomiting. Unsolicited

TABLE 2. Clinical Outcomes at Week 4 Post-FMT

Outcome	Week 4 Post-FMT, N (%)
Clinical Response	7 (35.0)
Clinical Remission	3 (15.0)
Mucosal Healing	2 (10.0)
Escalation of Therapy	3 (15.0)
Colectomy	1 (5.0)
Medical	2 (10.0)

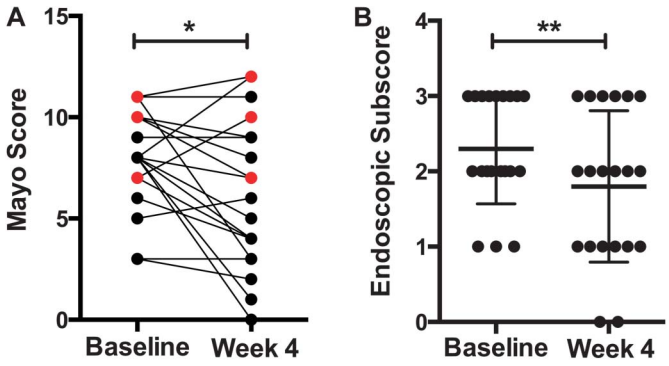

FIGURE 1. Two-donor FMP improved Mayo score and endoscopic subscore at week 4 post-FMT. A, Complete Mayo score at baseline and 4 weeks post-FMT. Red dots indicate subjects requiring escalation of care. B, Endoscopic subscore score at baseline and 4 weeks post-FMT. Wilcoxon matched-pairs signed-rank test, *P*-value *< 0.05, **<0.01 is indicated.

adverse events from patient-initiated telephone calls and office visit were also recorded. Adverse event severity and relatedness was graded using the FDA toxicity grading scale. Secondary outcomes included clinical response (ΔMayo score ≥3 and a bleeding subscore ≤1), clinical remission (Mayo score ≤2 and no subscore >1), and progression of disease (measured by initiation of anti-tumor necrosis factor α, escalation of dosage, or colectomy).

Lamina Propria Cell Isolation and Flow Cytometry

Lamina propria mononuclear cells were isolated from colonic tissue as previously described.[8] LIVE/DEAD fixable aqua dead cell stain kit (Molecular Probes) was used to exclude dead cells. For cytokine detection, cells were stimulated with phorbol myristate acetate and ionomycin with BD GolgiPlug for 4 hours. After surface-marker staining with anti-CD3-APCCy7 (eBiosciences UCHT1) and anti-CD4-BUV650 (BioLegend OKT4), cells were prepared as per manufacturer's instruction with Cytoperm/Cytofix (BD Biosciences) for intracellular cytokine evaluation of IL-17A (eBiosciences eBio64DEC17), IL-4 (eBiosciences 8D4-8), IL-22 (eBiosciences 22URTI), and IFNγ (eBiosciences 4S.B3). For transcription factor analysis, cells were fixed and permeabilized as per manufacturer's instructions (eBiosciences) and stained intracellularly with anti-Foxp3-E450 (eBiosciences 236A/E7) and anti-RORγt-PE (eBiosciences AFKJS-9). Data acquisition was computed with BD LSRFortessa flow cytometers and analysis performed with FlowJo software.

16S rRNA Gene Sequencing and Analyses

16S rRNA gene sequencing methods were adapted from the methods developed for the NIH-Human Microbiome Project.[9] Briefly, bacterial genomic DNA was extracted using MO BIO PowerSoil DNA Isolation Kit (MO BIO Laboratories). The 16S rDNA V4 region was amplified by PCR and sequenced on the

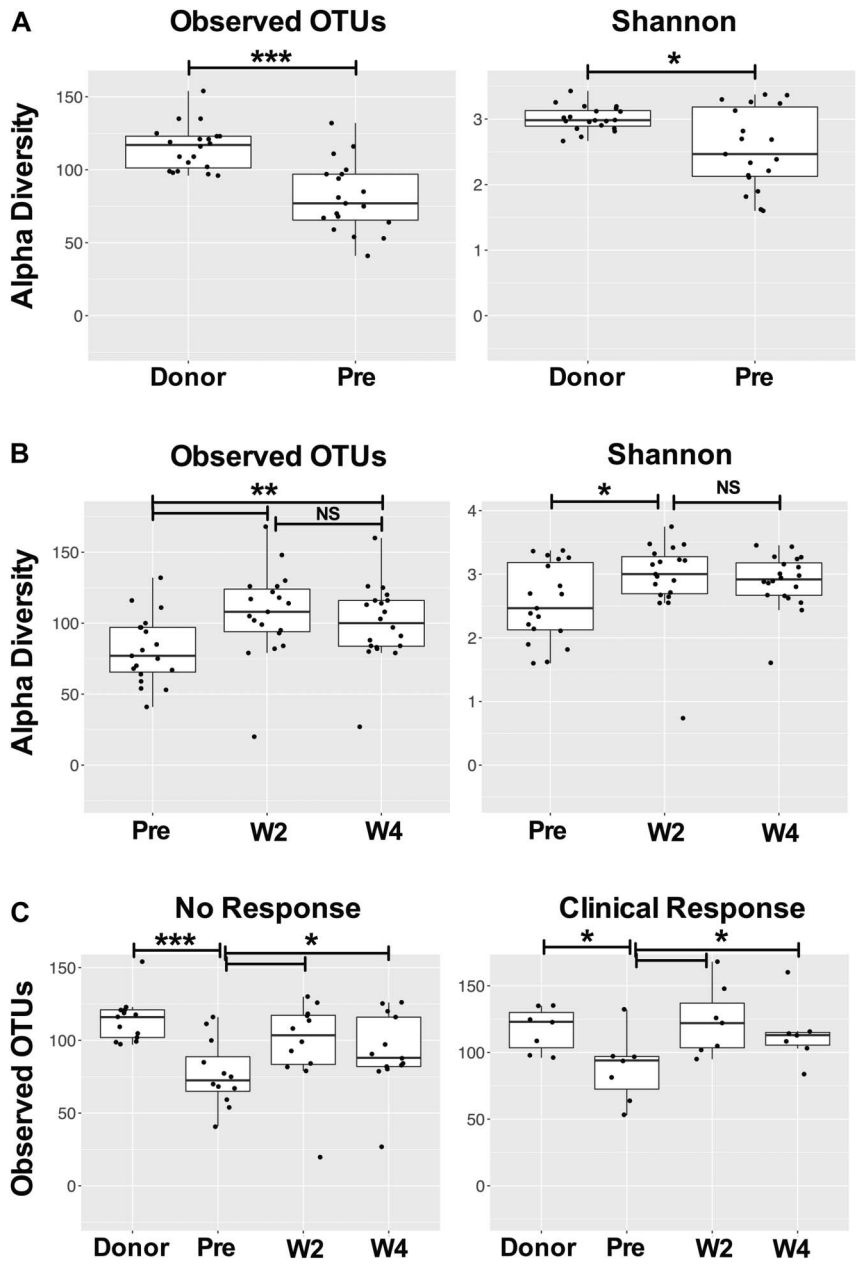

FIGURE 2. Two-donor FMP increases recipient diversity. Fecal DNA samples were sequenced by 16S rRNA sequencing. A, Alpha diversity metrics of observed OTUs (left panel) and Shannon index (right panel) are compared for donor and recipient pretransplant (pre). B, Alpha diversity metrics of observed OTUs (left panel) and Shannon index (right panel) are compared for recipient pretransplant (pre) and at week 2 (W2) and 4 (W4) after FMT. C, Alpha diversity of samples faceted by the primary endpoint of clinical response (No, left panel; Yes, right panel). For all panels *P* values are shown, Kruskal–Wallis. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

MiSeq platform (Illumina) using the 2 × 250 bp paired-end protocol.[10] The 16S rRNA gene pipeline incorporates phylogenetic and alignment-based approaches to maximize data resolution.[11] The read pairs were demultiplexed based on the unique molecular barcodes, and reads were merged using USEARCH v7.0.1090,[12] allowing zero mismatches and a minimum overlap of 50 bases. Merged reads were trimmed at the first base with Q5. In addition, a quality filter was applied to the resulting merged reads and reads with >0.05 expected errors were discarded. 16S rRNA gene sequences were clustered into Operational Taxonomic Units (OTUs) at a similarity cutoff value of 97% using the UPARSE algorithm. OTUs were mapped to an optimized version of the SILVA Database[13,14] containing only the 16S V4 region to determine taxonomies. Abundances were recovered by mapping the demultiplexed reads to the UPARSE OTUs. A rarefied OTU table (>8000 reads/sample) from the output files generated in the

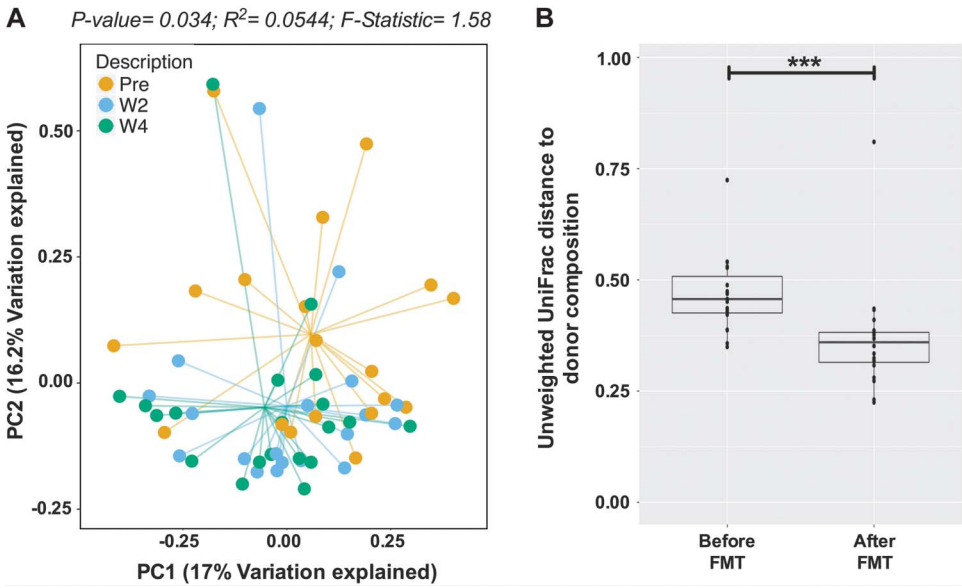

FIGURE 3. Two-donor FMP engrafts effectively in active UC recipient. A, Principal coordinate analysis plot is shown using Bray-Curtis dissimilarity matrix and stratified by recipient pretransplant (pre, orange) and at week 2 (W2, blue) and 4 (W4, green) after FMT. *P* values are shown, Monte Carlo, PERMANOVA. B, Unweighted UniFrac distance to donor is shown for the recipient before FMT and at 4 weeks post-FMT. ***$P < 0.001$, Wilcoxon signed rank.

previous 2 steps was used for downstream analyses of α-diversity, β-diversity,[15] and phylogenetic trends.

RESULTS

Two-Donor FMP Is Safe and Effective in Active UC

To increase the microbial diversity of the donor material, we prepared 2-donor FMPs. Simulation models using single donor sequence data (validated on sequence data from large 48-donor pools) reliably show that multiple donor FMP has greater diversity than single donor FMP (see Fig. S1, Supplemental Digital Content 1, http://links.lww.com/IBD/B504). Increasing pool size, however, simultaneously decreases FMP heterogeneity (i.e., difference in β diversity) (see Fig. S2, Supplemental Digital Content 1, http://links.lww.com/IBD/B504). Therefore, to maintain FMP heterogeneity and the possibility to detect differential donor effects resulting from unique microbial communities, 2-donor FMP was used in this study.

Baseline clinical characteristics for all 20 subjects are presented in Table 1. All 20 enrolled patients successfully completed the trial through week 4 (Table 2). Although no serious adverse events were observed, minor grade 1 adverse events were recorded, all of which were deemed to be "possibly related" to FMT (see Table S1, Supplemental Digital Content 1, http://links.lww.com/IBD/B504). One patient had a transient febrile response, which improved with conservative care within 5 days. Three patients required escalation of care after week 4 post-FMT. Two of these 3 patients had an increase in their week 4 Mayo score and were treated with either anti-tumor necrosis factor α therapy or colectomy. The third patient had a clinical response per study definition, but opted for anti-tumor necrosis factor α blockade therapy with a post-FMT Mayo score of 7. Consistent with previous studies using single donor FMP, 2-donor FMP was well tolerated in the setting of active UC.

Seven patients (35%) achieved a clinical response (ΔMayo score ≥3 and a bleeding subscore ≤1) by week 4. Three patients (15%) were in clinical remission at week 4 (Mayo score ≤2 and no subscore >1), and 2 of these patients (10%) achieved mucosal healing (endoscopy subscore of 0). Paired analysis showed a significant improvement in Mayo score (Median decrease of 1.5, $P = 0.03$) (Fig. 1A) and endoscopic subscore (Median decrease of 0.5, $P = 0.002$) (Fig. 1B). Mayo score improved significantly in biologic naive ($P < 0.001$), but not in biologic exposed patients (see Fig. S3, Supplemental Digital Content 1, http://links.lww.com/IBD/B504). No significant differences in ESR or CRP were seen post-FMT. All week 4 responders maintained a lower partial Mayo score at week 12 (see Fig. S4, Supplemental Digital Content 1, http://links.lww.com/IBD/B504). Of 13 nonresponders, 7 were tracked until week 12. Four achieved a partial Mayo score <2 and 3 had no change in the partial Mayo score at week 12. Collectively, these data suggest that single colonoscopic delivery achieved sustained clinical improvement, but longer follow-up will be needed to assess a potential delayed benefit of FMT.

Two-Donor FMP Effectively Engrafts in Both Responders and Nonresponders

To evaluate the impact of 2-donor FMP on microbial diversity, 16S rRNA gene sequencing was performed on recipient

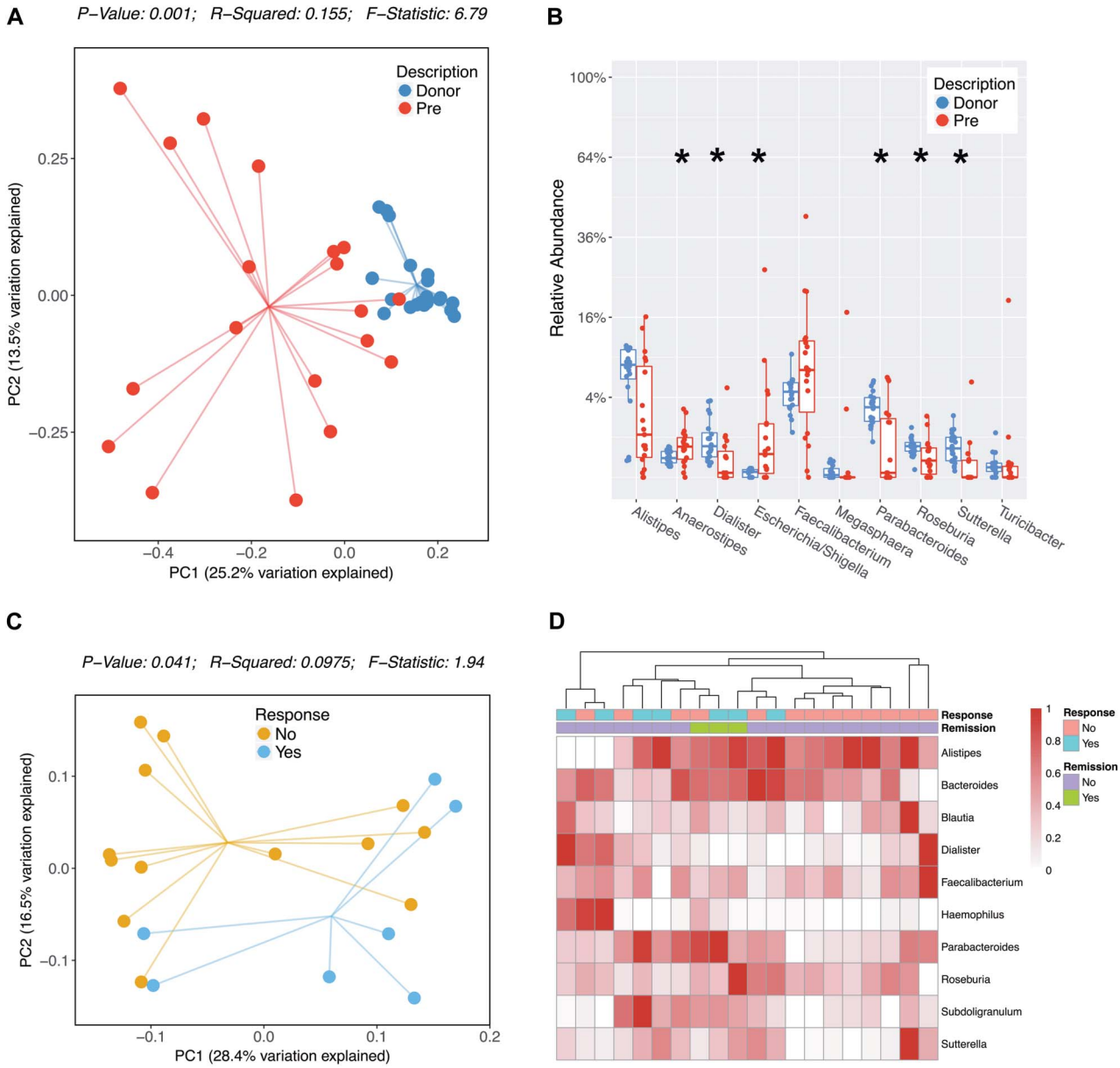

FIGURE 4. Donor composition correlates with clinical response. A, Principal coordinate analysis plot is shown using Bray–Curtis dissimilarity matrix and stratified by donor (blue) and recipient pretransplant (pre, red). *P* values are shown, Monte Carlo, PERMANOVA. B, Boxplot displays the top 10 differential abundant bacteria by genus between donor and recipient pretransplant (Pre). *P* values indicated by *<0.05, Mann–Whitney, FDR-adjusted. C, Principal coordinate analysis of donor composition by Bray–Curtis dissimilarity matrix stratified by clinical response. *P* values indicated, Monte Carlo, PERMANOVA. D, Hierarchical clustering of a heatmap representation of donor composition by genus colored by clinical remission or response as indicated. Scale represents the normalized relative abundance.

fecal DNA samples pretransplant and 2 and 4 weeks post-transplant. Analysis of species-level alpha diversity in pretransplant recipients revealed significantly fewer observed OTUs ($P = 4.1 \times 10^{-5}$) and a lower Shannon diversity index ($P = 0.03$) compared with the 2-donor FMP (Fig. 2A). Compared with recipient sample pretransplant, 2-donor FMP significantly enhanced OTU diversity at both 2 and 4 weeks post-FMT and Shannon diversity at 2 weeks post-FMT (Fig. 2B). This effect of 2-donor FMP was seen in both clinical responders and nonresponders (Fig. 2C). Although no differences were seen in either the donor sample or recipients pre-FMT, alpha diversity metrics at week 4 were significantly higher in the patients on corticosteroid/biologic/immunomodulator therapy (see Fig. S5, Supplemental Digital Content 1, http://links.lww.com/IBD/B504).

To evaluate engraftment as a function of microbial composition, we analyzed recipient beta diversity pre- and

post-transplant. Principal coordinate analysis of Bray-Curtis dissimilarity matrix revealed significant differences in the recipient pretransplant compared with 2 and 4 weeks post-transplant ($P = 0.034$) (Fig. 3A; see Fig. S6A, Supplemental Digital Content 1, http://links.lww.com/IBD/B504). Similarly, week 4 recipient samples were more similar to donor FMP than pretransplant recipient samples by unweighted UniFrac (Fig. 3B) and Jensen–Shannon (see Fig. S6B, Supplemental Digital Content 1, http://links.lww.com/IBD/B504) distance. These data suggest that a single colonoscopic delivery of 2-donor FMP is safe and effective in increasing microbial diversity in patients with active UC.

Donor Composition Correlates with Clinical Response

In addition to the significantly enhanced diversity of 2 donor FMP, principal coordinate analysis of Bray–Curtis dissimilarity matrix revealed significant differences between 2-donor FMP and pretransplant recipients (Fig. 4A). At the genus level, 2-donor FMP had higher levels of *Dialister*, *Parabacteroides*, *Roseburia*, and *Sutterella*, whereas recipients had higher levels of *Anaerostipes* and *Escherichia/Shigella* (Fig. 4B). Given these taxonomic differences between donor and recipient, we next evaluated donor microbial composition according to clinical response at week 4. Principal coordinate analysis of Bray–Curtis dissimilarity matrix revealed significant separation between donor samples achieving clinical response and those not (Fig. 4C). Although specific genus-level differences alone did not significantly differentiate these donor samples, donors achieving clinical response and remission clustered together on taxonomic alignment (Fig. 4D). Collectively, these data show that despite characteristic differences in donor FMP with active UC, sufficient heterogeneity exists in the 2 donor FMP to segregate donor microbial characteristics with clinical outcome.

Two-Donor FMP Alters Mucosal T-Cell Responses

Alterations in the composition of the intestinal microbiome have been shown to impact mucosal T-cell effector function in mouse models.[16,17] To evaluate the impact of 2 donor FMP on mucosal immunity in active UC, we profiled mucosal $CD4^+$ T-cell function in rectal biopsies performed at the time of FMT and 4 weeks post-FMT (see Fig. S7, Supplemental Digital Content 1, http://links.lww.com/IBD/B504). Analysis of $CD4^+$ T-cell cytokine production revealed a significant reduction in IFNγ production at 4 weeks post-FMT ($P = 0.02$) (Fig. 5A). No difference in IL-4, IL-17, or IL-22 was seen. Intranuclear staining of transcription factors FoxP3 or RORγt was performed to identify regulatory T cells (Treg) or T helper cells that produce IL-17 (Th17). Our results revealed a significant reduction in Tregs at week 4 compared with time of transplant ($P = 0.006$), but no difference in mucosal Th17 cells was seen (Fig. 5B). These data reveal the ability of 2-donor FMP to impact mucosal $CD4^+$ T-cell effector function.

DISCUSSION

This is the first study to evaluate the clinical, microbiological, and immunological impact of 2-donor FMP in FMT for active UC. Although this study was not placebo-controlled, clinical response (35%) and remission rates (15%) were similar to a recently reported placebo-controlled trial (39% response and 24% remission at week 7).[4] A recent multidonor, intensive FMT strategy requiring daily enema therapy showed even higher remission rates.[5] FMT with 2 donor FMP in our study was well tolerated, and the frequency of grade 1 adverse events was consistent with the expected incidence in both the treatment and controls reported previously.[3,4] These data, coupled with the significant decrease in week 4 Mayo score and endoscopic subscore, suggest that the 2 donor FMP is safe and effective. Similar to repeat enema delivery, these data support the short-term efficacy of single colonic delivery in achieving microbial engraftment. All the seven week 4 responders maintained improvements in clinical scores at week 12, but longer follow-up will be needed to evaluate the efficacy of less intensive therapy which may ultimately be more practical for patient care.

Given the restricted microbial diversity of a UC recipient with active disease, diversity and donor engraftment have been used as metrics of clinical efficacy. Increase in microbial

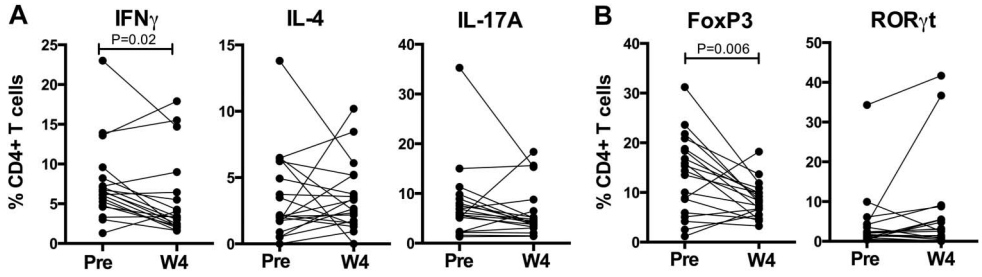

FIGURE 5. Two-donor FMP alters mucosal T-cell responses. Lamina propria mononuclear cells (LPMCs) were isolated from rectal endoscopic biopsies taken before (Pre) and 4 weeks after FMT (Post). A, LPMCs were stimulated with phorbol myristate acetate/ionomycin for 4 hours and intracellular cytokine staining was performed. The percentage of total $CD4^+$ T cells producing the designated cytokine is indicated. *P* values reflect paired T test. B, LPMCs were stained for Foxp3 and RORγt expression. The percentage of total $CD4^+$ T cells expressing the designated transcription factor is indicated. *P* values reflect paired T test.

diversity correlates with response in recurrent *C. difficile*-associated diarrhea.[1] While pilot studies of FMT in Crohn's disease[18] showed an increase in both diversity and donor engraftment, which correlated with clinical response, the correlation of diversity and engraftment with clinical response in UC was modest.[3,4] Using a 2-donor FMP to provide greater microbial diversity, our results reveal a robust increase in both diversity and engraftment in all subjects regardless of clinical response. These metrics reflect an overall improvement in clinical Mayo score and suggest that parameters in addition to microbial diversity contribute to clinical outcome.

Donor microbial characteristics, for example, may be critical in understanding clinical efficacy in UC. Most notably, the positive clinical results shown by Moayyedi et al are primarily driven by the success of FMT with "superdonor B."[4] Consistent with these results, our 2-donor composition strategy afforded us significant heterogeneity to segregate donor FMP that achieved clinical response by microbial community. Although no specific OTU was identified, donor microbiota inducing clinical remission showed enrichment in Parabacteroides, which has been shown to attenuate colitis in mice.[19]

The ability of FMT to impact the mucosal immune response remains a critical question. Although Crohn's disease was initially characterized as a Th1- and UC as a Th2-driven inflammatory disease, additional T-cell subsets have been identified to play a role in IBD mucosal barrier immunity including regulatory T cells and IL-23 responsive Th17 cells.[16] In mouse models, human microbiota have been shown to play a critical role in regulating Th1,[20] regulatory T cells,[21] and Th17.[17,22] Immune cell analyses in FMT studies have been limited, but a recent analysis in Crohn's disease revealed an increase in $CD25^+$ $CD127^{lo}$ $CD4^+$ T cells at 12 weeks post-FMT.[18] The reduction of Th1 and concomitant decrease in regulatory T cells that we report may reflect an acute reduction in inflammation consistent with the overall reduction in clinical and endoscopic scores. Longer follow-up is needed to evaluate potential long-lasting effects on mucosal immunity such as regulatory T-cell expansion.

This study has several limitations. First, our study was not placebo-controlled and endoscopists were not blinded. Although our study supports the safety and efficacy of single colonoscopic delivery of a high-diversity 2 donor FMP, recent studies have highlighted the role for repeat enemas[4] and intensive treatment[5] in achieving clinical outcome. Future studies are needed to evaluate the efficacy of less intensive, but potentially more practical, treatment strategies. Second, our results only provide short-term analysis of microbial engraftment and mucosal immunity, whereas clinical endpoints may require 8 to 12 weeks. Third, our study included patients with heterogenous disease on various medication backgrounds. Although this "real world" approach allowed us to capture robust week 4 clinical responses in biologic naive patients, larger placebo-controlled trials are needed to evaluate durable FMT strategies in both biological naïve and refractory patients. In addition, the impact of concurrent medical therapy on FMT warrants further evaluation.

FMT has exciting potential as therapy for UC. Our study provides proof-of-principle that 2 donor combinations can help to identify donor microbiota that drive clinical efficacy. Coupled with immune cell profiling, these approaches may help to identify critical, transferable, immune-reactive probiotics that may serve as the backbone for FMT in UC.

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REFERENCES

1. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med.* 2013;368:407–415.
2. Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology*. 2008;134:577–594.
3. Rossen NG, Fuentes S, van der Spek MJ, et al. Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology*. 2015;149:110–118. e114.
4. Moayyedi P, Surette MG, Kim PT, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology*. 2015;149:102–109. e106.
5. Paramsothy S, Kamm MA, Kaakoush NO, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *Lancet*. 2017;389:1218–1228.
6. Kump PK, Grochenig HP, Lackner S, et al. Alteration of intestinal dysbiosis by fecal microbiota transplantation does not induce remission in patients with chronic active ulcerative colitis. *Inflamm Bowel Dis.* 2013;19:2155–2165.
7. Kelly LB, Koelsch E, Dubois N, et al. *Prospective Laboratory Evaluation of Fecal Microbiota Transplantation Donors: Results From an International Public Stool Bank*. New Orleans, LA: ID Week; 2015.
8. Diehl GE, Longman RS, Zhang JX, et al. Microbiota restricts trafficking of bacteria to mesenteric lymph nodes by CX(3)CR1(hi) cells. *Nature*. 2013;494:116–120.
9. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486:207–214.
10. Caporaso JG, Lauber CL, Walters WA, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *Isme J.* 2012;6:1621–1624.
11. Buffington SA, Di Prisco GV, Auchtung TA, et al. Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. *Cell*. 2016;165:1762–1775.
12. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*. 2010;26:2460–2461.
13. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 2013;41:D590–D596.
14. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods.* 2013;10:996–998.
15. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol.* 2005;71:8228–8235.

16. Longman RS, Yang Y, Diehl GE, et al. Microbiota: host interactions in mucosal homeostasis and systemic autoimmunity. *Cold Spring Harb Symp Quant Biol*. 2013;78:193–201.
17. Viladomiu M, Kivolowitz C, Abdulhamid A, et al. IgA-coated E. coli enriched in Crohn's disease spondyloarthritis promote Th17-dependent inflammation. *Sci Transl Med.* 2017;9(376).
18. Vaughn BP, Vatanen T, Allegretti JR, et al. Increased intestinal microbial diversity following fecal microbiota transplant for active Crohn's disease. *Inflamm Bowel Dis.* 2016;22:2182–2190.
19. Kverka M, Zakostelska Z, Klimesova K, et al. Oral administration of Parabacteroides distasonis antigens attenuates experimental murine colitis through modulation of immunity and microbiota composition. *Clin Exp Immunol.* 2011;163:250–259.
20. Mazmanian SK, Liu CH, Tzianabos AO, et al. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell*. 2005;122:107–118.
21. Atarashi K, Tanoue T, Oshima K, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*. 2013;500:232–236.
22. Tan TG, Sefik E, Geva-Zatorsky N, et al. Identifying species of symbiont bacteria from the human gut that, alone, can induce intestinal Th17 cells in mice. *Proc Natl Acad Sci U S A.* 2016;113:E8141–E8150.