Alteration of Intestinal Dysbiosis by Fecal Microbiota Transplantation Does not Induce Remission in Patients with Chronic Active Ulcerative Colitis

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Background: In patients with ulcerative colitis (UC), alterations of the intestinal microbiota, termed dysbiosis, have been postulated to contribute to intestinal inflammation. Fecal microbiota transplantation (FMT) has been used as effective therapy for recurrent Clostridium difficile colitis also caused by dysbiosis. The aims of the present study were to investigate if patients with UC benefit from FMT and if dysbiosis can be reversed.

Methods: Six patients with chronic active UC nonresponsive to standard medical therapy were treated with FMT by colonoscopic administration. Changes in the colonic microbiota were assessed by 16S rDNA–based microbial community profiling using high-throughput pyrosequencing from mucosal and stool samples.

Results: All patients experienced short-term clinical improvement within the first 2 weeks after FMT. However, none of the patients achieved clinical remission. Microbiota profiling showed differences in the modification of the intestinal microbiota between individual patients after FMT. In 3 patients, the colonic microbiota changed toward the donor microbiota; however, this did not correlate with clinical response. On phylum level, there was a significant reduction of Proteobacteria and an increase in Bacteroidetes after FMT.

Conclusions: FMT by a single colonoscopic donor stool application is not effective in inducing remission in chronic active therapy-refractory UC. Changes in the composition of the intestinal microbiota were significant and resulted in a partial improvement of UC-associated dysbiosis. The results suggest that dysbiosis in UC is at least in part a secondary phenomenon induced by inflammation and diarrhea rather than being causative for inflammation in this disease.

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Key Words: inflammatory bowel disease, ulcerative colitis, fecal microbiota transplantation, stool transplantation, intestinal microbiota, 16S rDNA, microbial community profiling

Inflammatory bowel diseases (IBD), including Crohn’s disease and ulcerative colitis (UC), are chronic inflammatory conditions of the intestine resulting from an inappropriate immune response to luminal antigens.12 The cause of the chronic activation of the immune system is currently unknown. Endogenous enteric bacteria, also termed the intestinal microbiota, can directly interact with the gastrointestinal immune system of the human host.2–5 In genetically susceptible hosts, specific changes in the composition of the intestinal microbiota, called dysbiosis, can lead to activation of the mucosal immune system resulting in chronic inflammation of the intestine in IBD.2–5 Nevertheless, it is questionable whether dysbiosis itself causes the disease or if it represents an epiphenomenon due to microbial alterations as a consequence of IBD.6

The major hallmarks of UC therapy include the use of anti-inflammatory and immunosuppressant drugs1–7; however, some patients fail to respond to medical therapy and require total proctocolectomy to control inflammation and complications of the disease.7 In therapy-refractory recurrent Clostridium difficile infection (CDI), an intestinal disease also linked to dysbiosis, fecal microbiota transplantation (FMT) was recently demonstrated to be a highly effective and safe therapy.8–14 This therapy aims at restoring a normal intestinal microbiota by intestinal application of filtered and diluted stool from healthy subjects. This form of therapy has also been applied in patients with IBD. Up to date, 8 patients suffering from UC successfully

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treated with FMT have been published, in two case reports and a small series of patients. In these reports, clinical and endoscopic remission was observed in all patients after a period of 6 weeks, resulting in the discontinuation of anti-inflammatory medications. All patients experienced a sustained remission ranging from 6 months to 13 years. So far there are, however, no reports investigating changes in the intestinal microbiota due to FMT in UC or the extent to which donor bacteria can colonize the colon of the recipient in humans. It is also unknown whether FMT in patients with IBD can result in reversal of IBD-associated dysbiosis.

The aim of the present study was to investigate the benefit of FMT for patients with therapy-refractory UC by assessing the clinical and endoscopic response. The secondary aim was to study changes in the intestinal microbiota after FMT by 16S rDNA-based microbial community profiling.

### PATIENTS, MATERIALS AND METHODS

**Patients**

This study included 6 patients, between July and December 2011, suffering from chronic active UC refractory to conventional medical therapy, who were considered for total colectomy as a last resort (Table 1). Diagnosis of UC was established by standard clinical, endoscopic, and histologic criteria. Intestinal superinfections due to *C. difficile*, *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, and cytomegalovirus were excluded by stool culture, toxin enzyme immunoassay, or by immunohistochemistry in all patients before inclusion in the study. Further exclusion criteria were pregnancy, age less than 16 years, and participation in other clinical studies. Patients younger than 18 years required a guardian’s consent.

**TABLE 1. Patient Characteristics at Baseline, Previous and Ongoing Therapies**

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Sex</th>
<th>Duration of Disease (yr)</th>
<th>Montreal Classification</th>
<th>Mayo Score</th>
<th>Previous Therapy</th>
<th>Ongoing Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A</td>
<td>39</td>
<td>M</td>
<td>12</td>
<td>E3</td>
<td>10</td>
<td>Azathioprine 5-ASA Prednisolon Infliximab Adalimumab 5-ASA (3 g/d) 5-ASA (40 mg/wk)</td>
</tr>
<tr>
<td>Patient B</td>
<td>17</td>
<td>F</td>
<td>3</td>
<td>E3</td>
<td>8</td>
<td>Azathioprine 5-ASA Prednisolon Infliximab Adalimumab 5-ASA (3 g/d) 5-ASA (15 mg/d)</td>
</tr>
<tr>
<td>Patient C</td>
<td>31</td>
<td>M</td>
<td>3</td>
<td>E3</td>
<td>10</td>
<td>5-ASA Prednisolon Infliximab Adalimumab 5-ASA (4 g/d) 5-ASA (100 mg/d)</td>
</tr>
<tr>
<td>Patient D</td>
<td>45</td>
<td>F</td>
<td>3</td>
<td>E3</td>
<td>11</td>
<td>5-ASA Prednisolon Infliximab Adalimumab 5-ASA (4 g/d) 5-ASA (20 mg/d)</td>
</tr>
<tr>
<td>Patient E</td>
<td>52</td>
<td>M</td>
<td>2</td>
<td>E2</td>
<td>10</td>
<td>Azathioprine 5-ASA Prednisolon Infliximab Adalimumab 5-ASA (2 g/d) 5-ASA (4 g/d)</td>
</tr>
<tr>
<td>Patient F</td>
<td>35</td>
<td>F</td>
<td>10</td>
<td>E2</td>
<td>8</td>
<td>Azathioprine Methotrexate 5-ASA Prednisolon Infliximab 5-ASA (4 g/d) 5-ASA (2 g/d)</td>
</tr>
</tbody>
</table>

5-ASA, 5-aminosalicylic acid.
Donors
The intended stool donors were 4 healthy adults (mean age, 40 years; range, 26–59 years; 3 women and 1 man), nonrelatives living in a different household to the recipients. Donors had received no antibiotic therapy within the past 6 months. To avoid a transmission of other diseases, donors had to have a negative history for intestinal diseases or recent gastrointestinal infections, autoimmune or other immune-mediated diseases, or any kind of malignancies. Chronic hepatitis B and C, human immunodeficiency virus, cytomegalovirus, and syphilis were excluded serologically and the donor’s stool was tested for C. difficile, enterohemorrhagic Escherichia coli, Salmonella, Shigella, Yersinia, and Campylobacter as well as for parasites and helminths. Stool samples of the donors were collected at enrollment for further analysis of the microbiota. Two donors provided stool for 2 patients, one donor (donor AC) for patients A and C and another donor (donor EF) for patients E and F with an interval of 2 and 3 weeks between donations, respectively. The donor for patient B (donor B) and the donor for patient D (donor D) provided stools only for these patients.

Preparation of Donor Stool
Donors underwent a mild colonic lavage using polyethylene glycol 3350 (PEG 3350) before stools were collected in special vessels (2500–mL vessels for disposable specimens; LP Italiana Spa, Milano, Italy) and stored at 4°C. Within the next 6 hours, the stool of a single bowel movement weighing 100 to 150 g was diluted with sterile normal saline (200–350 mL) and filtered through sterile gauze twice to remove crude components. A total of 300 to 500 mL of the extracted suspension containing the donor’s intestinal flora was placed into 20-mL syringes. An aliquot of the original donor stool was frozen at enrollment for further analysis of the transferred microbiota.

Protocol for FMT and Follow-up of Patients
At enrollment (day 0, baseline), the patient underwent a rectosigmoidoscopy without any previous bowel lavage or enema. Tissue samples were collected from the sigmoid colon at about 30 cm above the anal canal for histology and analysis of the mucosa-adherent microbiota. Intestinal biopsies and stool samples used for microbiota analysis were immediately stored at −80°C. Furthermore, stool calprotectin was determined and blood samples for blood count and for routine blood chemistry were obtained. At the follow-up visits at days 1, 7, 30, and 90 after FMT, we repeated these procedures, and fecal specimens and colonic biopsies were obtained for microbiota analysis (Fig., Supplemental Digital Content 1, http://links.lww.com/IBD/A220). Three samples were missing in the data set for microbiota analysis: day 90 fecal and mucosal sample of patient C because the patient underwent total colectomy after day 30 and the fecal samples of patient C on day 1 as the patient had no bowel movement on this day.

FMT was performed on the day following baseline (day 0a). After bowel lavage using a standard PEG solution (Moviprep), the patients underwent colonoscopy. Starting from the terminal ileum, fractions of 20 mL of the donor’s fecal solution were applied into the ileum and colon through the biopsy channel of the colonoscope. A total of 300 to 500 mL of diluted and filtered bacterial suspension was transferred into the patient’s colon and terminal ileum. About two thirds of the bacterial suspension was installed into the ileum and the right colon, the rest into the transverse and left colon. After the procedure, patients received 4 mg of loperamide to slow intestinal transit and allow accurate time for colonization of donor bacteria. The patients were instructed to keep their regular diet as before the procedure.

During control visits, the clinical condition and endoscopic activity of UC were judged using the Mayo score. A reduction of the total Mayo score by ≥3 points was considered as a clinical response, whereas a drop of the Mayo score to ≤2 points was considered as remission.

DNA Extraction, 16S rDNA Amplification, and Pyrosequencing
Stool samples and biopsies from patients and donors were immediately frozen and stored at −20°C before DNA isolation. An aliquot of 175 mg of the stool samples and a total single colonic biopsy were homogenized in a MagnaLyser Instrument (Roche Diagnostics, Mannheim, Germany), and bacterial DNA was isolated with the MagNA Pure LC DNA Isolation Kit III (bacteria, fungi) in a MagNA Pure LC 2.0 Instrument (Roche Diagnostics) according to the manufacturer’s instructions. The enzyme cocktail II with 100 μg of lysozyme per 100 μL of sample was used according to the manufacturer’s instructions.

Bacterial 16S rDNA was amplified using FLX 454 1-way read fusion primers (Lib-L kit, Primer A, Primer B; Roche 454 Life Science, Branford, CT) with the template-specific sequence F27—AGAGTTTGATCCTGCGCTAG and R534—ATTACCGCGGCTGCTGGC targeting the V1–V3 hypervariable region of the 16S rRNA gene (Table, Supplemental Digital Content 2, http://links.lww.com/IBD/A221). For each sample, a PCR mix of 50 μL was prepared containing 1× Fast Start High Fidelity Buffer (Roche Diagnostics), 2.5 U of High Fidelity Enzyme (Roche Diagnostics), 200 μM deoxynucleotide triphosphates (Roche Diagnostics), 0.4 μM barcoded primers (Eurofins MWG, Ebersberg, Germany), PCR-grade water (Roche Diagnostics), and 62.5 ng of total (fecal or biopsy) genomic DNA. Thermal cycling conditions were for initial denaturation at 95°C for 3 minutes followed by 30 cycles of denaturation at 95°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 1 minute with a final extension of 7 minutes at 72°C. Amplicons were purified and collected using a denaturing HPLC on a WAVE apparatus (Transgenicom, Inc, Omaha, NE) and eluted using a linear gradient (typically 12%–17%) of acetonitrile in 0.1 M triethylammoniumacetate over 10 minutes at 50°C. Amplicon DNA was washed on NucleoFast 96 PCR plates (Macherey-Nagel, Düren, Germany) with 70% ethanol and eluted in 30 μL of elution buffer (Macherey-Nagel). Amplicon DNA concentrations were determined using the Quant-it PicoGreen dsDNA Assay Kit (Life Technologies, Carlsbad, CA) according to the
Phylogenetic and Biostatistical Analyses

After initial quality filtering with Qiime23 (read length > 200, no ambiguous bases, minimum quality score of 25, homopolymer run < 7) 184,666 high-quality 16S rDNA sequences with an average length of 515 bp (range, 200–899 bp) remained. For the assignment of operational taxonomic units, reads were clustered with uclast24 allowing for 3% sequence distance (97% sequence identity threshold). Taxonomy was assigned by using the RDP classifier version 2.5.25 Contaminant (human) sequences and singletons were manually removed. Evenness, the Shannon diversity index and richness estimators Chao1 and abundance-based coverage estimator were calculated using the R package BiodiversityR.26 Weighted UniFrac distance27 was calculated with Qiime. Principal coordinate analysis on the normalized, log-2 transformed data was performed by Qiime. Taxa significantly changing between time-points were assessed by Metastats using default settings.28 For the statistical analysis and significant difference calculation, the nonparametric Wilcoxon test for dependent samples was used.

ETHICAL CONSIDERATIONS

The study was approved by the local institutional review board (Medical University of Graz Ethic Committee, EK-number 23-357 ex 10/11, approved on June 7, 2011), and written informed consent was obtained from all patients and the stool donors. The study protocol was published in the German register of clinical trials (DRKS00003824; Deutsches Register klinischer Studien, www.germanctr.de).

RESULTS

Patient Characteristics

The clinical history and the baseline characteristics of the 6 patients with chronic active therapy–refractory UC are reported in Table 1. The average age of the patients was 36 years, and the mean duration of UC before inclusion in the study was 5 years. All patients had already been treated with anti–tumor necrosis factor α (TNF-α) antibodies. All except one patient (patient C) had received at least one additional immunosuppressive medication. Medications at the time of enrollment, including anti–TNF-α antibodies (1 of 6 patients) and 5-aminosalicylic acid (5 of 6 patients), were maintained throughout the study period at stables doses. Steroids (4 of 6 patients) were further tapered down during the first 30 days of the study (Table 1).

Safety of FMT

Patient A, who additionally received anti-TNF treatment, had an increase in stool frequency and developed fever up to 39°C 1 day after FMT. Laboratory tests showed a temporary elevation of C-reactive protein (31.5 mg/L; normal, <5.0 mg/L) and interleukin-6 (14.2 pg/mL; normal, <7.0 pg/mL). The fever was interpreted as an immunological reaction to the applied bacteria into the inflamed colon. Because the patient was clinically not severely affected and symptoms of colitis did not deteriorate, it was considered as a nonsignificant side effect. The fever was self-limited and ceased after 3 days without any further treatment. Neither elevated body temperature nor elevated C-reactive protein levels were observed in any other patient. No other adverse events occurred after FMT or during the follow-up period of 3 months.

Clinical and Endoscopic Response to FMT

None of the patients achieved remission within the 90-day follow-up interval after FMT as indicated by the Mayo score (Fig. 1A). However, all patients reported a temporary improvement within the first 2 weeks after FMT, referring to a decrease of stool frequency at day 7 (Fig. 1B, P = 0.031). Stool frequency increased again on day 30 in 4 of 6 patients. Two patients had a sustained clinical improvement up to day 90 after FMT evidenced by a reduction of 3 and 4 points in the total Mayo score (patient A and F, respectively; Fig. 1A). There was no change in the fecal calprotectin levels (Fig., Supplemental Digital Content 3, http://links.lww.com/IBD/A222) or serum C-reactive protein levels. Patient B experienced a relapse of UC and underwent total colectomy, after initial temporal improvement. Patients C, D, and E showed no significant change in the Mayo score. The clinical outcome of nonresponders was the performance of total proctocolectomy in 2 patients (patients B and D) and treatment with calcineurin inhibitors in 2 patients (cyclosporine A in patient C and tacrolimus in patient E).

After 1 year of clinical follow-up, 3 patients still have chronic active UC. Patient A is participating in a clinical trial using a new biologic agent, patient C is treated with azathioprine and intermittent corticosteroids, and patient E is treated with 5-aminosalicylic acid and intermittent corticosteroids. Patient F is in clinical remission on 5-aminosalicylic acid monotherapy.

Changes of the Intestinal Microbiota After FMT

On average, 2374 reads (range, 636–6581) were generated per sample. A total of 6539 operational taxonomic units (OTUs) (97% ID) were assigned, with an average of 394 (patient - stool), 289 (patient - mucosa), and 334 (donor - stool) OTUs per individual. Most sequences were related to the phyla Firmicutes
Bacteroidetes (18.3%), Proteobacteria (18.3%), Fusobacteria (4.5%), Tenericutes (2.9%), and Actinobacteria (1.4%). We found no significant difference in the relative abundance of phyla between mucosal and fecal samples (Fig. 2). A total of 64 bacterial families were found in the samples, 12 of them accounted for more than 85% of the OTUs in the whole data set (Table, Supplemental Digital Content 4, http://links.lww.com/IBD/A223).

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FIGURE 1. Clinical response of patients and development of microbiota richness after FMT. Each graph shows the individual values for each patient as well as the median values before (day 0) and after FMT (days 1, 7, 30, 90) for the Mayo score (A), maximal stool frequency (B), bacterial richness for mucosal (C) and stool samples (D).

FIGURE 2. Composition of the colonic microbiota in patients before and after FMT and composition of the donor’s microbiota on phylum level. In patients, pooled fecal and mucosal samples at different time points are provided separately. There was a progressive decrease in Proteobacteria and an increase in Bacteroidetes in fecal and in mucosal samples after FMT. Proteobacteria: mucosa baseline: 29%, mucosa day 1: 23% (§P = 0.77), mucosa day 7: 13% (§P = 0.18), mucosa day 30: 10% (%P = 0.071), mucosa day 90: 7% (\&P = 0.041); stool baseline: 33%, stool day 1: 35% (<P = 0.81), stool day 7: 17% (>P = 0.068), stool day 30: 6% (#P = 0.041), stool day 90: 2% (*P = 0.024); Bacteroidetes: mucosa baseline: 4%, mucosa day 1: 17% (§P = 0.78), mucosa day 7: 18% (§P = 0.087), day 30: 25% (%P = 0.031), mucosa day 90: 27% (\&P = 0.035); stool baseline: 6%, stool day 1: 13% (<P = 0.48), stool day 7: 18% (>P = 0.075), stool day 30: 27% (#P = 0.078), stool day 90: 23% (*P = 0.027). The reported P-values are for comparison with baseline samples.
**Bacterial Richness, Diversity, and Evenness**

FMT was associated with a temporal increase in species richness in fecal and mucosal samples of the patients; however, this trend failed to reach statistical significance. Species richness, as defined as the number of different OTUs represented in a sample, peaked at day 1 (day 0: 394 ± 273 versus day 1: 497 ± 108; P = 0.63) in fecal samples and at day 7 (day 0: 251 ± 122 versus day 7: 349 ± 98; P = 0.16) in mucosa samples after FMT (Fig. 1C, D) and thereafter decreased to baseline. The Shannon diversity index, a measure of the bacterial diversity within a sample and encompassing richness and evenness of OTUs, peaked at day 7 (day 0: 3.62 ± 0.76 versus day 7: 4.27 ± 0.42; P = 0.31) in mucosal samples and decreased thereafter (Table, Supplemental Digital Content 5, http://links.lww.com/IBD/A224). In fecal samples, no trend toward an increased diversity could be observed after FMT (Table, Supplemental Digital Content 6, http://links.lww.com/IBD/A225). The temporal increase in bacterial richness and diversity after FMT on the mucosa was observed in all patients except patient D (Fig. 1C, D). Donor stool samples did not differ to patient’s baseline stool samples in regard to richness and diversity (Fig. 1D, and Table, Supplemental Digital Content 6, http://links.lww.com/IBD/A225).

**Changes in the Composition of the Colonic Microbiota After FMT**

We observed 4 different patterns of changes in the colonic microbiota of recipients after FMT (Fig. 3, and Fig., Supplemental Digital Content 7, http://links.lww.com/IBD/A226); (1) a convergence of the patient’s microbiota toward the donor’s microbiota at days 30 and 90 (patients B, C, and E); (2) a convergence of the patient’s microbiota to the donor’s microbiota on day 30 followed by a reversal to the baseline patient microbiota on day 90 (patient A); (3) changes in the patient’s microbiota neither approaching the donor’s microbiota nor the baseline microbiota (patient D); (4) the microbiota of patient F at baseline was very similar to the donor’s microbiota and thereafter the observed changes were minor as compared with the other patients. Noteworthy, patient F showed the best clinical response to FMT. To assess whether the recipient microbiota approached toward the donors microbiota after FMT, we compared the similarity of the individual microbiota by using the weighted UniFrac distance. The UniFrac distance is a measure of similarity (i.e., decreased distance) or dissimilarity (i.e., increased distance) when individual samples are compared. Our analyses revealed that in patients B, C, and D, the UniFrac distance of the samples after FMT compared with the respective donor was lower than the distance of the samples after FMT compared with the respective baseline samples. Thus, individual microbiotas in patients B, C, and D resembled more the microbiota of the donor after FMT (Fig. 4, and Fig., Supplemental Digital Content 7, http://links.lww.com/IBD/A226). Overall, we observed large differences in the individual microbiotas after FMT as compared with baseline samples except for patient F (Fig. 4, and Fig., Supplemental Digital Content 7, http://links.lww.com/IBD/A226).

**Altered Microbial Community After FMT**

FMT lead to a considerable change in the composition of the colonic microbiota on phylum level in fecal and in mucosal samples. After FMT, the relative abundance of Proteobacteria decreased significantly from baseline to day 90 in mucosal and fecal samples (mucosa: 29% versus 7%, P = 0.048; stool: 33% versus 2%, P = 0.024) (Fig. 2). The decrease of Proteobacteria on the mucosa 30 days after FMT fell short of statistical significance. Complementarily, the relative abundance of Bacteroidetes increased significantly from baseline to day 30 in mucosal samples and to day 90 in mucosal and fecal samples (Fig. 2). The decrease of Proteobacteria was pronounced in patients B, C, D, and E, whereas patients A and F had low levels of Proteobacteria at baseline (total 1.3% and 1.4%, respectively) and therefore showed no considerable changes except for a transient increase at day 1 in patient A (Fig. 3).

On family level, we noted significant relative changes in Bacteroidaceae, Enterococcaceae, Turicibacteraceae, Clostridiales Family XIII, and Enterobacteriaceae after FMT (Tables, Supplemental Digital Contents 9 and 10, http://links.lww.com/IBD/A227, http://links.lww.com/IBD/A228, and http://links.lww.com/IBD/A229). In total, levels of 3 of the 12 most abundant families changed significantly (Tables, Supplemental Digital Contents 4, 9, and 10, http://links.lww.com/IBD/A223, http://links.lww.com/IBD/A228, and http://links.lww.com/IBD/A229). The most prominent relative changes were a decrease in Enterobacteriaceae and Enterococcaceae and a relative increase in Bacteroidaceae. The family of Enterobacteriaceae represented 63% of all Proteobacteria in the samples.

Other taxa that have been reported to be underrepresented in IBD, such as the family of Lachnospiraceae, increased numerically but not significantly in relative abundance (stool samples baseline: 11% versus day 90: 21%; P = 0.33).

**DISCUSSION**

In our study, we were unable to reproduce the promising results of previous reports using FMT for the treatment of UC. None of our patients achieved remission, and only 2 patients showed clinical improvement of colitis within the 90 days follow-up after a single FMT. To date, the effect of FMT on the colonic microbiota...
FIGURE 3. Principal coordinate analysis of donor stool (green circles) and stool (purple circles) and corresponding mucosal samples (blue circles) of the individual patients (patients A, B, C, D, E, and F) before (day 0) and after FMT (days 1, 7, 30, and 90). The principal components 1 and 2 accounting for up to 45% variability are shown. The bars on the right panels show the composition of the mucosal and stool microbiota as percentage of all bacterial classes represented at each time point for the patients and the corresponding donor’s microbiota.
in IBD has not been extensively studied in detail. We observed marked changes in the composition of the intestinal microbiota after FMT as determined by 16S rDNA-based microbial community profiling, in contrast to the minor clinical effects.

The direct analysis of the human intestinal microbiota has been hampered for long time by the fact that the majority of gut microorganisms cannot be cultivated. Only an estimated 10% to 20% of gut microbes can be assessed using standard cultivation techniques. This challenge was overcome by PCR amplification of phylogenetic markers (e.g., 16S rDNA) in combination with high-throughput sequencing techniques that enabled culture-independent surveys of human gut microbes. Using these techniques, remarkable changes in the human gut microbiota in patients with IBD have been observed. These changes, also termed dysbiosis, are thought to be involved in the pathogenesis of IBD. Dysbiosis in patients with IBD, reflecting a dominance of potentially pro-inflammatory pathogens or pathobionts over anti-inflammatory commensals, is thought to result in a shift of the immunological balance in the intestinal mucosa toward inflammation. It still remains unclear whether the observed dysbiotic changes in the microbiota of patients with IBD are primarily responsible for the activation of the immune system and subsequent inflammation or if they rather represent a consequence of colitis and diarrhea caused by altered bacterial growth conditions.

In our study, we could observe a reversal of some of the reported dysbiotic changes in the intestinal microbiota of patients with UC after FMT, including a marked decrease in Proteobacteria including Enterobacteriaceae, an increase in Bacteroidetes, and a concomitant increase of Firmicutes and Actinobacteria in tissue or fecal samples. Furthermore, changes in the abundance of lower taxonomic ranks, such as an increase in the family of Enterobacteriaceae, have been reported in patients with UC.

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In our study, we could observe a reversal of some of the reported dysbiotic changes in the intestinal microbiota of patients with UC after FMT, including a marked decrease in Proteobacteria including Enterobacteriaceae, an increase in Bacteroidetes, and a concomitant increase of Firmicutes and Actinobacteria in tissue or fecal samples. Furthermore, changes in the abundance of lower taxonomic ranks, such as an increase in the family of Enterobacteriaceae, have been reported in patients with UC. Finally, in advanced and long persisting UC reversal of dysbiosis and the introduction of anti-inflammatory bacteria by FMT might fail to decrease gut inflammation because of a persisting and overwhelming immune stimulation. Changes of the intestinal microbiota after FMT have so far been studied only in recurrent CDI.
Successful FMT in CDI was associated with an increase in bacterial diversity and richness, an increase of Bacteroidetes, Firmicutes, and butyrate-producing bacteria such as Lachnospiraceae, and a decrease in Proteobacteria. Similar changes have been observed in our study. Dysbiosis and the corresponding changes after FMT are at least in part similar in recurrent CDI and UC; their causative role might be, however, different in these 2 diseases. It should be noted that other previously reported alterations of the microbiota in patients with UC, such as an increase in Actinobacteria, were not found in our patients’ samples at baseline or during follow-up.

The lack of efficacy of FMT in most of our patients with UC compared with the previous series might be explained by several factors. We performed only a single application of diluted donor stool that was administered to the ileum and colon without antibiotic pretreatment. A single donor stool administration has been in most protocols for the treatment of recurrent CDI. Bennett and Brinkman and Borody et al. used antibiotic pretreatment in their reported patients with UC although studies in mice showed that this may not be necessary for successful colonization of the donor’s microbiota and may only result in a reduced bacterial diversity after FMT. Borody et al. performed repeated administration of donor stool per rectal enema for 5 days in contrast to our single administration. In 3 of our patients (B, C, and E), the intestinal microbiota approached the donor’s microbiota after FMT (Fig. 3). These patients, however, showed no better clinical response as compared with the other patients, implying that colonization by the donor’s microbiota is not the sole critical factor for clinical response. Beside the protocol for FMT, the composition of the donor’s microbiota is likely an important factor influencing the clinical success. So far, there is no established strategy on how to screen donors for potential “beneficial” or anti-inflammatory bacteria rather than choosing healthy individuals without recent antibiotic therapy and lacking intestinal or autoimmune diseases. It is possible that the microbiotas of our donors were not suitable for inducing anti-inflammatory effects in UC. The fact that an identical donor was used for the patient with the best clinical response (patient F) and also for another patient who did not improve (patient E) challenges this possibility. Moreover, fecal microbiotas of patients E and F showed marked similarities on day 90 after FMT (Fig. 3, and Fig., Supplemental Digital Content 8, http://links.lww.com/IBD/A227). Finally, patient selection might be a factor influencing the response to FMT. In our series, all patients had a therapy–refractory long-lasting disease course and were considered for colectomy. In the previously reported series, patients also had chronic active long-lasting disease, and previous therapies included immunosuppressive therapy in at least 4 of the patients; however, no patient was treated with anti-TNF antibodies. It remains, therefore, possible that FMT in milder forms of UC without previous anti-TNF therapy is more effective.

Some observations in our series of patients are of special interest and might lead to a better understanding of which microbial factors could lead to a successful therapeutic effect. Patient F had a sustained clinical response to FMT and is in clinical remission on long-term follow-up without additional immunosuppressive therapy. Although this could be a spontaneous remission of UC, it is noticeable that the composition of the donor’s microbiota was very similar to the patient’s microbiota at baseline. Moreover, the patient’s microbiota contained only low levels of Proteobacteria and high levels of Bacteroidetes, especially Prevotellaceae, compared with the other patients (Fig. 3). Studies in mice have shown that a successful intestinal colonization by pathogens but also by beneficial bacteria are linked to the presence of phylogenetically similar bacteria already present in the intestinal ecosystem. It might therefore be important for successful FMT that the microbiota of the recipient contains related bacteria to the donor’s microbiota, however, with a different profile regarding anti-inflammatory properties. All our patients showed an improvement of their symptoms, especially with regard to diarrhea, which lasted for up to 2 weeks after FMT. During this period, we found a temporal increase in phylotype richness and diversity on the mucosa, which was not present thereafter when diarrhea worsened again. If increased bacterial richness is one important microbial factor influencing disease activity in patients with UC, FMT protocols resulting in a sustained increase in bacterial richness might be more successful. Some of the observed changes in the intestinal microbiota might be induced by bowel preparation using a PEG solution before colonoscopy. In a previous article, we could demonstrate that osmotic diarrhea induced by PEG results, however, in an increase of Proteobacteria and a decrease in the richness and diversity of the intestinal microbiota, which is opposite to the observed changes in the microbiota of patients with UC after FMT in the current study. This makes a significant long-term effect of bowel preparation for this study very unlikely. Short-term changes in the microbiota, as the marked increase of Proteobacteria in patient A on day 1 after FMT (Fig. 3), which was not observed at later time points, may be indeed an effect of bowel preparation.

We have examined only a small number of patients without a control group. Nevertheless, we believe that the information gained by our data will help to better understand dysbiosis and its role in the pathogenesis of IBD because there are no other published studies investigating the effect of FMT on the intestinal microbiota in IBD. The discrepancy between correction of some prominent dysbiotic features, such as a pronounced decrease in Proteobacteria induced by FMT despite the lack of clinical efficacy in our pilot trial, is an important finding because the overabundance of Proteobacteria is the most consistent finding of dysbiosis in UC and Crohn’s disease reported so far. We also could demonstrate that the donor’s microbiota can colonize the colon, at least in some patients with UC, successfully. Future studies will need to focus also on investigating bacterial function (i.e., metagenomic and metatranscriptomic analyses) of intestinal microbiota besides taxonomic description. Further trials of FMT in patients with UC will need to address if other characteristics of dysbiosis and their correction will lead to a successful treatment of UC.

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