Case Report

PGHN

Failure of Fecal Microbiota Transplantation in a Three-Year-Old Child with Severe Refractory Ulcerative Colitis

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Fecal microbiota transplantation (FMT) is a treatment designed to correct gut dysbiosis by administration of feces from a healthy volunteer. It is still unclear whether FMT for children with ulcerative colitis (UC) is effective or hazardous. Here we describe a young patient to have received FMT for UC. A three-year-old girl was admitted to our hospital with severe active UC, and treated with aminosalicylates and various immunosuppressive drugs. As remission was not achieved, we decided to try FMT before colectomy. We administered donor fecal material a total of six times by retention enema (×2) and via a nasoduodenal tube (×4) within 10 days. The patient developed abdominal pain and pyrexia after each FMT session. Analyses revealed the transferred donor fecal microbiota had not been retained by the patient, who ultimately underwent colectomy. The severity of the UC and/or timing of FMT may have partly accounted for the poor outcome.

Key Words: Colectomy, Fecal microbiota transplantation, Gastrointestinal microbiome, Inflammatory bowel diseases, Pediatric ulcerative colitis

INTRODUCTION

Fecal microbiota transplantation (FMT) is an investigational treatment designed to correct dysbiosis in the gut by administration of feces from a healthy volunteer. The gut microbiota contributes to harvest of energy from food, and changes in the gut microbiome may be associated with some bowel diseases [1]. Ulcerative colitis (UC) is a chronic relapsing inflammatory disorder of the colon. Although the cause remains unknown, immunological abnormalities triggered by genetic and environmental factors are thought to play an important role in its pathogenesis [2]. Current medical treatments for UC include aminosalicylates, thioprines, corticosteroids, tumor necrosis factor-modulating agents, and other immunosuppressives [3]. Some patients with UC require colectomy because of poor response to these treatments. Recently a randomized control study has reported that FMT can have therapeutic benefits for

adult patients with UC [4]. However, it is still unclear whether FMT for children with UC is effective or hazardous.

Here we report a three-year-old girl with refractory severe UC for whom colectomy ultimately became necessary after sequential FMT proved ineffective. Fecal analysis revealed that the donor's microbiota had failed to become established in the gut of the patient. In this case, the severity of the UC and/or the timing of FMT may have affected the outcome.

CASE REPORT

A three-year-old girl presented with a 3-month history of hematochezia that had gradually worsened. She was diagnosed as having severe active UC endoscopically and histologically, and treatment with aminosalicylates and steroids was started. Two weeks later she was referred to our hospital because of steroid refractoriness. She discharged a large volume of bloody diarrhea more than 20 times per day, and suffered marked abdominal pain, worsening fatigue and weight loss (from 12 kg at the time of onset to 10 kg). Blood examination demonstrated anemia (hemoglobin, 8.4 g/dL), leukocytosis (white blood cells, 39,500/μL), and elevated values of inflammatory markers (erythrocyte sedimentation rate, 16 mm/h,; C-reactive protein, 2.2 mg/dL). Abdominal radiography showed a paucity of bowel gas in the entire colon. Stool cultures and stool tests for toxins A and B all gave negative results. The Pediatric Ulcerative Colitis Activity Index (PUCAI) score was 85 (i.e., the maximum score possible).

For re-evaluation the patient underwent esophagogastroduodenoscopy and colonoscopy. Endoscopy revealed severe diffuse and continuous mucosal inflammation with superficial ulcers and erythema involving the entire colon (Fig. 1A), whereas the upper gastro-intestine and terminal ileum were intact. The histological features were compatible with UC, including acute colonic inflammation with crypt abscesses throughout the colon (Fig. 1B), without abnormalities in the esophagus, stomach, duodenum, and terminal ileum.

Because patients with very early-onset inflammatory bowel disease, diagnosed ≤5 years of age, frequently present with a severe phenotype and carry variants in genes associated with immunodeficiency, we analyzed the whole exome sequence of the patient and her parents using genomic DNA extracted from lymphocytes. However, no significant variants including the genes related to immunodeficiency were detected.

As the data from these investigations led us to reconfirm that the patient had severe UC, in addition to mesalazine and intravenous prednisolone, we administered parenteral nutrition from a central venous catheter and started the patient on intravenous cyclosporine A. However, these treatments did not alleviate the symptoms. We started the patient on infliximab at 6 mg/kg and scheduled further infliximab infusions, two and six weeks later. During this period we switched intravenous prednisolone and cyclosporine A to oral tacrolimus treatment. Since her daily bowel movements with bloody stools showed no significant change, and she required blood transfusions repeatedly because of anemia, we discontinued the infliximab infusions. Neither leukocytapheresis nor granulocytapheresis was indicated because of the patient's small body size. During the course of the above treatments, the patient received probiotic therapy with *Clostridium butyricum* (Miya BM; Miyarisan Pharmaceutical Co., Ltd., Tokyo, Japan). This probiotic is used safely in Japan for over 40 years. Recently, it has been reported that *C. butyricum* may induce interleukin-10-producing immunoregulatory macrophages and have potential as a safer therapeutic option for gut inflammatory diseases [5].

We discussed the available options with the patient's mother and guardians, and these included colectomy and unconventional medical therapy including FMT. They decided that FMT should be attempted before opting for colectomy. After obtaining permission from our institutional Bioethics Committee (approved number: Shin 15-02), written informed consent for FMT was obtained from the patient's
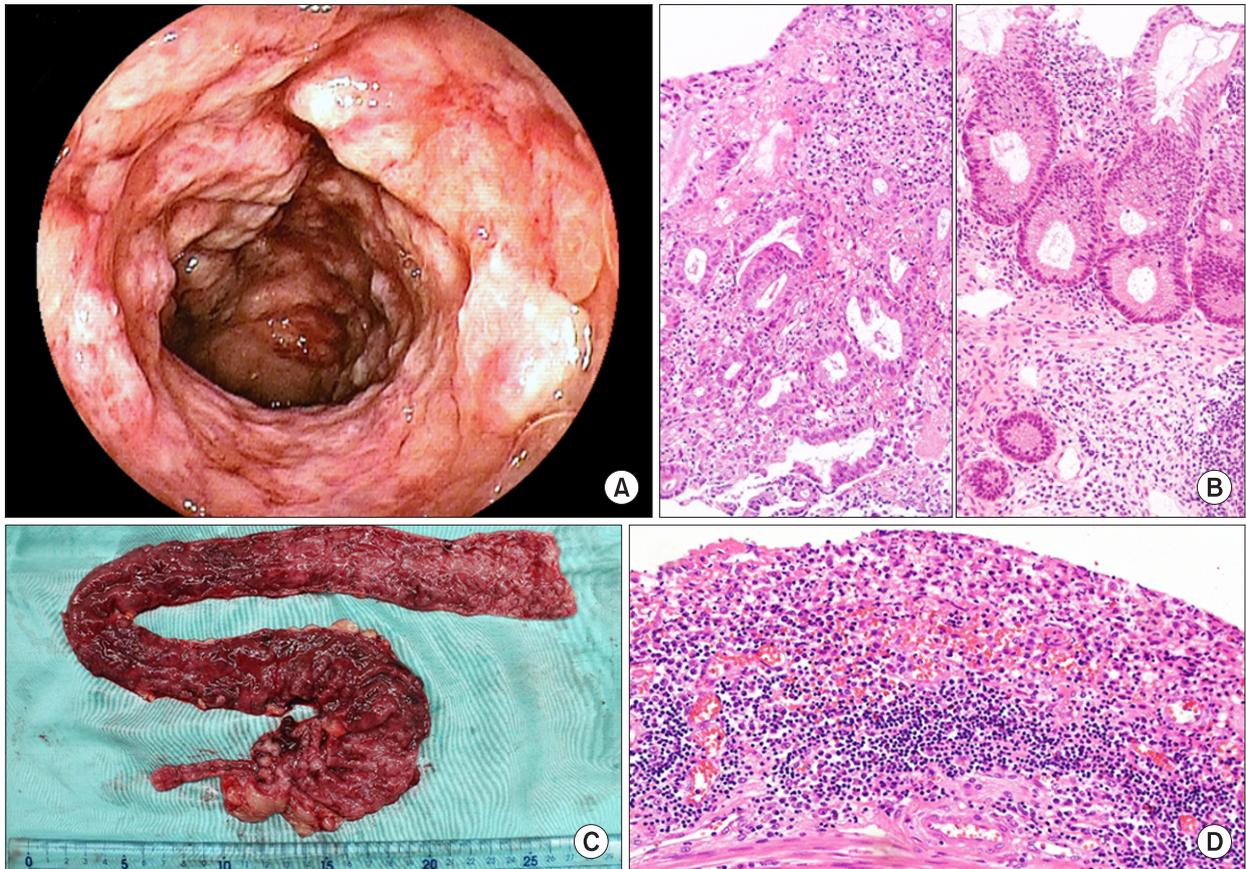
Fig. 1. (A) Colonoscopy demonstrates severe diffuse and continuous mucosal inflammation with superficial ulcers and erythema. (B) Low-power view of histological features in the colonic biopsy specimen compatible with ulcerative colitis, including active inflammation with cryptitis (hematoxylin and eosin [H&E] stain, ×100). (C) Gross appearance of a colectomy specimen, demonstrating diffuse and continuous severe mucosal inflammation and ulcerations with a partial pseudo-polyp-like appearance. (D) Medium-power view of a section of the colon, demonstrating severe active inflammation with loss of crypts and epithelium (H&E stain, ×200).

mother and guardians.

Donor selection

The patient's mother was chosen as a donor for FMT, and she was screened for infection risks according to the FMT workgroup recommendations [6]. Additional exclusion criteria included a history of any gastrointestinal diseases, use of antibiotics in the preceding three months, and immunosuppressive medications.

Donor stool preparation [6]

Collected donor stool material was used within three hours after collection. About 60 g of stool mate-

rial was added to sterile normal saline (250 mL) in a sterile container. Mixing was performed using a blender. The stool mixture was then filtered through gauze and aspirated into 60-mL slip tip syringes.

Donor stool administration

FMT was performed a total of six times using a retention enema (two times) and a flexible nasoduodenal tube (four times) within 10 days. We used the former method initially because the patient was young and considered incapable of tolerating endoscopic administration. We administered a 60 mL enema every 15 minutes with the patient in the left lateral decubitus position and the hips elevated [6].

However, during the retention enema, a small amount of leakage was unavoidable. Furthermore, there was no change in the patient's symptoms. Since her entire colon was involved, we performed FMT using 60-mL aliquots of fecal suspension delivered via a nasoduodenal tube every 15 minutes from the third to sixth sessions. Accordingly, we administered a total of 1,500 mL (250 mL×6 times) of donor stool liquid by retention enema (2 times) and via a nasoduodenal tube (4 times) within a 10-day period.

Side effects

After FMT on every occasion, abdominal pain and pyrexia occurred, and this improved over the following day. Although bacteremia was a considered a risk, blood bacterial culture tests revealed no detectable bacterial pathogens.

Stool sampling and microbiota analyses

Composition of the fecal microbiota was carried out by terminal restriction fragment length polymorphism (T-RFLP). DNA was extracted from fecal samples according to the method described by Matsuki et al. [7] with some modifications. Briefly, fecal samples were disrupted with glass beads using FastPrep-24 (MP Biomedicals, Irvine, CA, USA), then DNA was extracted with phenol. The extracted DNA was purified using a High Pure PCR Kit (Roche Diagnostics, Indianapolis, IN, USA). T-RFLP analysis was carried out as described elsewhere [8-10]. The primers used for polymerase chain reaction (PCR) amplification of the 16S rRNA gene were 27F and 35F labeled with 6-FAM, and 1492R. PCR products purified using a High Pure PCR Product Purification Kit (Roche Diagnostics, Penzberg, Germany) were digested with *HhaI* and *MspI*, and the digests were analyzed using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Fragment sizes of terminal restriction fragments (T-RFs) were estimated using GeneMapper Software (Applied Biosystems). Assignment of T-RFs and clustering of T-RFLP patterns were analyzed using Microbiota Profiler (Infocom, Tokyo, Japan).

Stool sampling was performed on the recipient's

stools three days before the first FMT, on the donor's stools used for the first FMT, and on the recipient's stools two days after the last FMT.

Fecal microbiota analyses revealed that the patient did not show a coherent composition resembling that of the healthy donor before treatment. After FMT, the patient's fecal microbiota composition was quite different from that of the donor. Therefore, it appeared that the transferred donor fecal microbiota had not been retained stably in the patient (Fig. 2).

The patient ultimately underwent colectomy three months after admission. The diagnosis of UC was reconfirmed on the basis of both macroscopic and microscopic examination of the removed colon (Fig. 1C, 1D).

DISCUSSION

The clinical course of this child illustrated two notable issues: first, that a pediatric patient with severe refractory active UC may not respond to FMT, and second, therapy using both a nasoduodenal tube and retention enema has been tolerated in such a young child, although transient fever and abdominal pain developed in this case.

In the present pediatric patient, comparison of the fecal microbiota composition between the donor and recipient revealed failure of the patient's intestine to retain the donor microbiota, despite the use of sequential FMT. One reason for the poor response might have been the severity of the disease. Previous reports of successful outcomes associated with FMT for UC involved patients with relatively mild disease [4,11,12]. In contrast, our patient had a short disease duration of seven months, and had received several different immunosuppressive drugs, including steroids, cyclosporin A, tacrolimus, and infliximab, reflecting the progressive course of the illness. The stratification between the outer mucus layer of the large intestine and the luminal contents is important from the standpoint of host microbial mutualism [13]. In severe UC cases, the outer layer of mucus might be influenced by a decreased number of in-
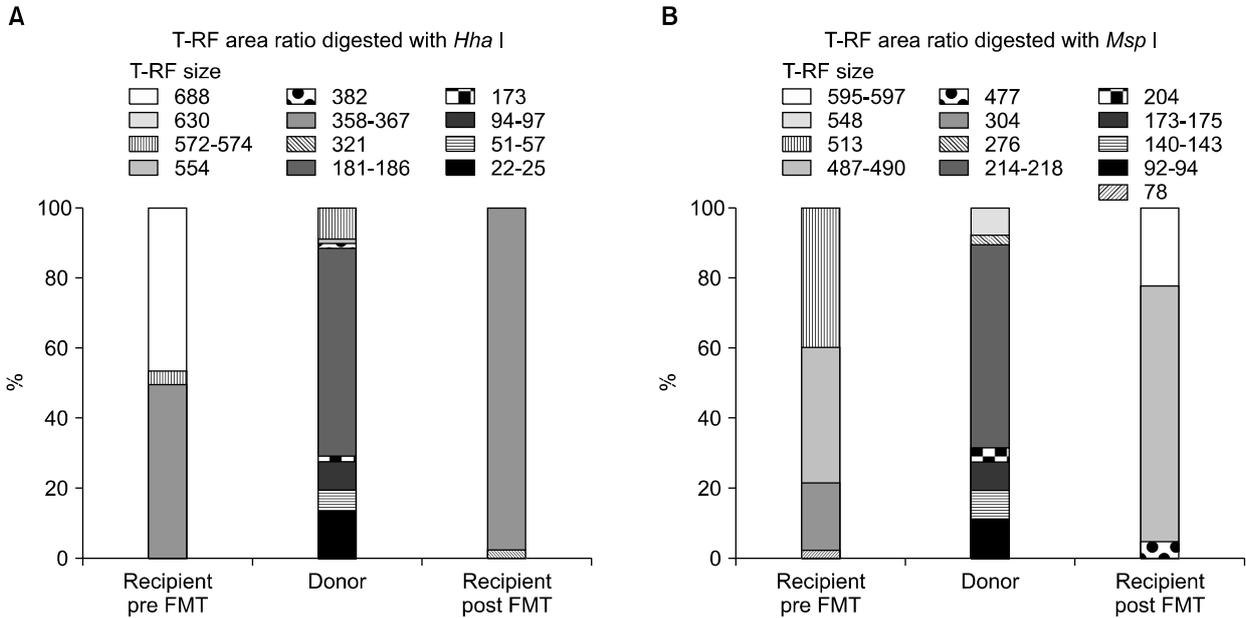
A

B

Fig. 2. Fecal microbiota composition of the donor and recipient measured by T-RFLP (A, T-RF area ratio digested by *HhaI*; B, T-RF area ratio digested by *MspI*). The patient underwent probiotic therapy with *Clostridium butyricum* throughout the treatment until colectomy. The graphs demonstrate that the patient did not show a coherent microbiota composition compared to the healthy donor before treatment. After fecal microbiota transplantation (FMT), the patient's fecal microbiota composition was quite different from that of the donor. Thus, the transferred donor fecal microbiota was not retained in the patient's gut. T-RF: terminal restriction fragments, T-RFLP: T-RF length polymorphism.

testinal goblet cells and increased intestinal motility. In our patient, the pathohistological features of the removed colon demonstrated a marked decrease in the number of crypts in the lamina propria and a severely damaged epithelium, both of which suggested a marked decrease of mucus in the outer layer. The patient also had hyperactive bowel movement. Thus, the donor's microbiota would have merely passed through the recipient's gut following the sequential FMTs, thus accounting for the discrepancy of the intestinal microbiota profiles between the donor and the patient after FMT. Angelberger et al. [14] reported that out of five adult patients who underwent FMT because of moderately to severely active UC, only one showed a positive clinical response after 12 weeks. Their findings tend to support this interpretation. Therefore, any pediatric patient with UC who has achieved remission through conventional medical therapy, or who has mild disease, might be a better candidate for FMT.

Another possible reason for the poor response might have been related to stool preparation and the method of administration for FMT. Vandenplas et al. [15,16] reported a transient positive response to FMT in a child who presented with early onset of an UC-like condition. Although they administered approximately 10 mL/kg of a 50% suspended fecal slurry, we decided to inject 25 mL/kg of a 20% preparation according to the FMT workgroup recommendations [6]. They administered the donor stool preparation using colonoscopy and a nasoduodenal tube. Endoscopic administration into the cecum is thought to be more effective than use of a retention enema; however, this patient was considered incapable of tolerating endoscopic administration. On the other hand, donor stool infusion through a nasoduodenal tube is thought to be more effective than use of a nasogastric tube because the former route means that microorganisms are unaffected by gastric acid. Therefore, we infused our stool preparation via a na-

soduodenal tube, as reported by Vandenplas et al. [15].

Although FMT delivered via both a nasoduodenal tube and retention enema had been tolerated, transient fever and abdominal pain developed. This suggests a temporary systemic immune response to FMT in children with UC. Similarly, Vermeire et al. [17] reported fever and abdominal tenderness in patients with Crohn's disease who underwent FMT. Vandenplas et al. [15] also reported transient symptoms including profuse sweating, vomiting, paleness, tachycardia, and fever. Bacteremia following FMT has also been reported [18], but in our patient the transient immune response was apparently not caused by bacteremia, as blood cultures were negative, although translocation of bacterial compounds such as lipopolysaccharide through disruption of the intestinal mucosal barrier caused by severe inflammation cannot be excluded [14].

FMT for severe refractory active UC may be challenging, and many unresolved issues remain, such as selection of the ideal donor, the most effective preparation method, the most ideal route of administration, and the most effective schedule or duration of FMT for inducing and maintaining a positive clinical response. Further investigation is needed to determine the optimal patient and donor selection, and the ideal FMT protocol for UC.

ACKNOWLEDGEMENTS

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