BPR MCS Bulletin of Pioneering Researches of Medical and Clinical Science

Bulletin of Pioneering Researches of Medical and Clinical Science

Available online: https://bprmcs.com
2022 | Volume 2 | Issue 2 | Page: 106-117

Induction of IL-10-Secreting, Filtrate-Reactive T Cells Following Sterile Fecal Microbiota Transplantation in Ulcerative Colitis

Sung Min Kim[1]*, Jae Hoon Lee[2], Soo Min Lee[3]

[1]Department of Clinical Medicine, Yonsei University College of Medicine, Seoul, South Korea.
[2]Department of Medical Sciences, Kyoto University, Kyoto, Japan.
[3]Department of Clinical Sciences, National Taiwan University, Taipei, Taiwan.

Abstract

Ulcerative colitis is a long-lasting, immune-driven condition of uncertain origin, occurring across various age groups and markedly impairing daily functioning. Current therapeutic strategies predominantly rely on anti-inflammatory agents and do not specifically target the underlying pathogenic mechanisms. In this work, we examined how transferring a sterile filtrate of stool from healthy individuals influences the activation of anti-inflammatory immune pathways. We found that this intervention in ulcerative colitis patients led to the emergence of T helper cells in circulation that recognized components of the sterile filtrates in an antigen-dependent fashion and secreted IL-10. In contrast, cells obtained from the same individuals prior to treatment responded weakly to these filtrates and mainly released IL-4, reflecting a more pro-inflammatory profile. These findings indicate that transplantation of a sterile stool filtrate promotes a shift toward an anti-inflammatory T-helper phenotype, an effect that strengthened and remained detectable for at least three months following the procedure.

Keywords: Ulcerative colitis, Sterile fecal microbiota transplantation, Regulatory T cells, Immune response

Corresponding author: Sung Min Kim
E-mail: smkim@gmail.com

How to Cite This Article: Kim SM, Lee JH, Lee SM. Induction of IL-10-Secreting, Filtrate-Reactive T Cells Following Sterile Fecal Microbiota Transplantation in Ulcerative Colitis. Bull Pioneer Res Med Clin Sci. 2022;2(2):106-17. https://doi.org/10.51847/Rcy3hWJ8ut

Introduction

Ulcerative colitis (UC) is a complex disorder primarily associated with excessive activation of the immune system. Yet no single pathogen or definitive antigenic trigger has been identified. UC pathogenesis is largely driven by immune-mediated inflammation arising from hypersensitivity processes in the subepithelial layers of the colon. Unlike Crohn's disease, UC is dominated by Th2-oriented immune responses, aligning it more with type 4 hypersensitivity reactions than with the Th17-dependent, bacteria-focused responses typically observed in Crohn's disease. This pattern involves heightened activity of CD4+ T cells that recognize specific antigens, which may or may not be microbial. Nevertheless, Th2-biased immunity is also a common feature of persistent chronic inflammation, including autoantigen- and microbe-related responses.

Currently, treatment options consist solely of anti-inflammatory pharmaceuticals, ranging from relatively mild non-steroidal agents to potent hormonal or cytostatic drugs. Despite advances brought by newer immunosuppressive treatments, the overall rate of stable remission remains low [1]. One possible explanation is that the heightened immune activity may not be the fundamental initiator of inflammation [2], meaning that inhibition of a single pathway may be insufficient, as

alternative immune routes can be triggered by unidentified factors. It is well established that inflammatory bowel diseases (IBD) involve insufficient regulatory T-cell activity [3, 4], placing them within the autoimmune spectrum due to impaired suppression of self-directed immune responses. Still, no reliable autoantigen with prognostic or therapeutic value has been identified.

A more recent perspective suggests that disturbances in the gut microbiome may drive these immune abnormalities. The intestinal microbial community acts as a key immune-educational environment [5], providing both innate signals (PAMPs, MAMPs) and antigenic inputs derived from bacteria [6]. A characteristic microbiome pattern in UC is an increase in the Firmicutes phylum relative to Bacteroidota, commonly revealed by 16S rRNA profiling [7]. Moreover, Bacteroidota species have been directly linked to the induction of T regulatory cells [8], while Firmicutes are often associated with autoimmune diseases such as rheumatoid arthritis [9, 10]. However, other research questions the usefulness of the Firmicutes/Bacteroidota ratio for IBD classification [11].

Fecal Microbiota Transplantation (FMT) has been proposed as a therapeutic approach for IBD. The original rationale was that transferring the gut microbial community from healthy donors might reduce inflammation by introducing bacteria capable of promoting immune regulation. Nonetheless, clinical studies have not provided definitive evidence of consistent benefit in UC or Crohn's disease patients [12]. Additionally, FMT is usually contraindicated in individuals with severe disease, limiting available data. Poor outcomes may arise because donor and recipient microbiomes differ substantially [13] or because the metabolic environment of the patient does not support successful microbial engraftment [14]. Overall, FMT appears most beneficial in milder cases or for extending remission in chronic UC [11].

A growing line of evidence suggests that the therapeutic effect of FMT may rely less on bacterial transfer and more on metabolites or bacteriophages present in donor material [15–17]. Contrary to the assumption that living bacteria must directly modulate immunity, bacteria-free approaches focus on regulatory impacts independent of engraftment. In animal models of dextran sulfate-induced colitis, FMT has been shown to alter T-helper cell balance toward regulatory and IL-10-producing subsets [18]. In human IBD studies, FMT has been associated with increased IL-10 production and reduced IL-17 levels in blood [19]. Some authors attribute these effects to successful colonization by donor microbiota and enhanced metabolite production that promotes regulatory T-cell differentiation. Other studies, however, argue that microbial colonization is not essential for clinical benefit [12].

The purpose of this study was to evaluate whether sterile stool preparations—completely depleted of bacterial cells—can modulate the activation of anti-inflammatory T-helper responses in patients following experimental Sterile Fecal Microbiota Transplantation (SFMT).

Results and Discussion

Patients

Eight adult individuals **(Table 1)** who met the study inclusion criteria and consented to receive sterile intestinal microbiota transplantation were enrolled. Their ages ranged from 20 to 51 years, and all had verified diagnoses of moderate or moderate-to-severe UC lasting longer than one year **(Table 1)**. Diagnosis was established using fecal calprotectin measurements, fibrocolonoscopy with anesthesia, and histological analysis of biopsy material collected throughout the colon and ileum. UC severity was determined by the Mayo scale, which evaluates stool frequency, visible blood, endoscopic appearance of the mucosa, and an overall clinical assessment. Scores of 2–5 or 6 indicated moderate or moderate-to-severe disease, respectively. Each participant also underwent routine blood testing, biochemical profiling (including total protein, urea, and bilirubin), and C-reactive protein measurement **(Table 1)**.

Table 1. Characteristics of UC patients enrolled in the study.

Patient	Gender	Age (Years)	Mayo Score	Fecal Calprotectin (μg/g)	C-Reactive Protein (mg/L)	Daily Bowel Movements	Blood in Stool	Hemoglobin (g/L)
1	Male	32	4	600	0.89	2–4	Present	125
2	Female	30	2	180	1.59	1	Absent	117
3	Female	30	5	600	1.67	2–4	Minimal	130
4	Male	34	4	600	6.45	4	Minimal	128
5	Female	22	2	46	0.68	4	Minimal	121
6	Male	22	6	1056	5.93	20	Present	133
7	Female	20	6	901	3.27	10	Present	92

8	Female	51	6	563	4	5	Present	117

Individuals with severe UC were not included to maintain a uniform study group and because such patients typically receive intensive immunosuppressive therapy, which could confound immune-related outcomes. Moreover, severe UC may carry a higher risk of worsening inflammation due to heightened immune reactivity.

Immune status of biological samples and experimental settings

The central assumption of the study was that administering sterile stool filtrates might generate an immunomodulatory effect driven by antigenic or adjuvant components originating from the healthy donor microbiota. Because direct assessment of intestinal T cells is technically difficult—and essentially impossible in patients with mild UC—we evaluated the activation and memory state of peripheral T cells. This was performed using peripheral blood mononuclear cells stimulated with sterile filtrates derived from donor stool and from patient stool collected at several time points. We examined CD4+ lymphocyte subsets and concentrations of IL-4 and IL-10, two cytokines involved in regulating inflammatory responses. Biological samples were obtained at baseline and at 1 and 3 months after transplantation **(Figure 1a)**.

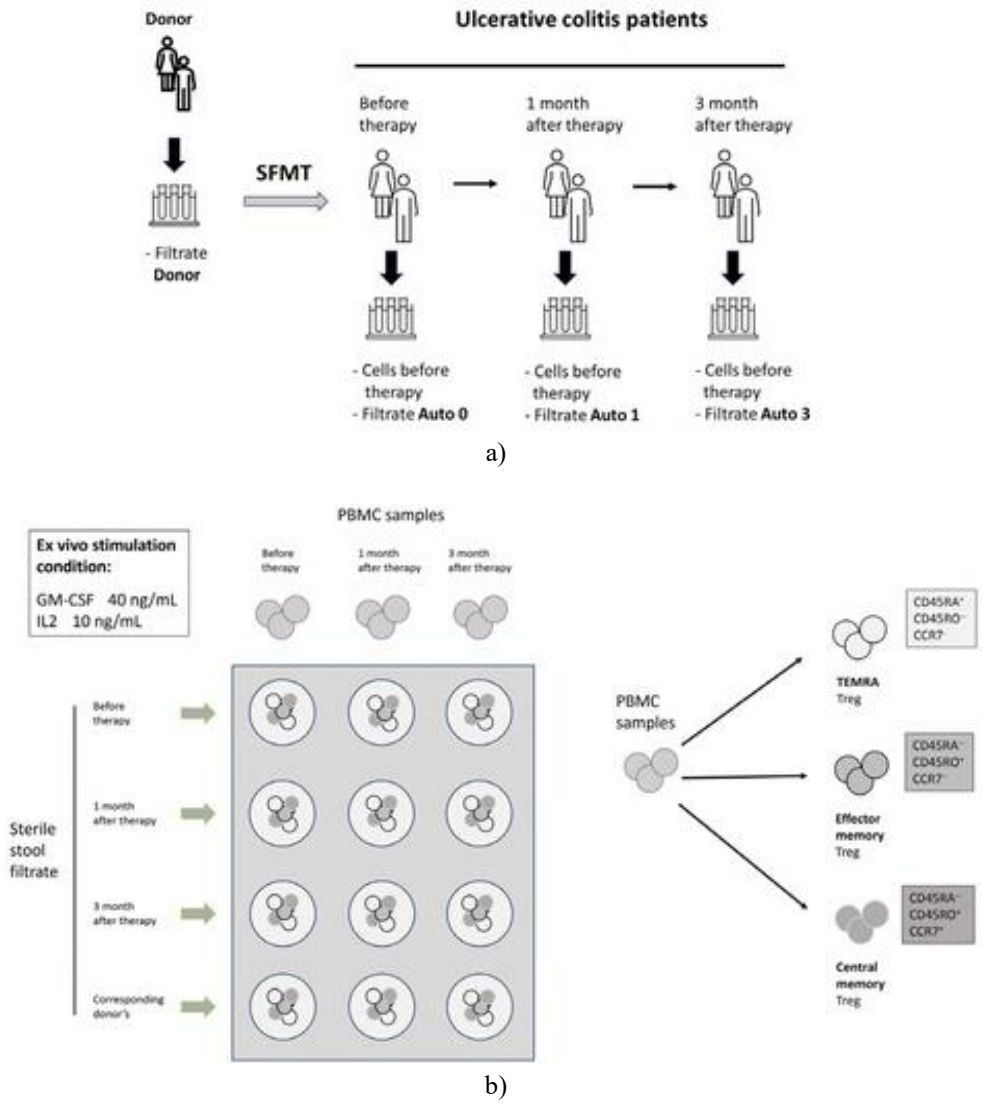
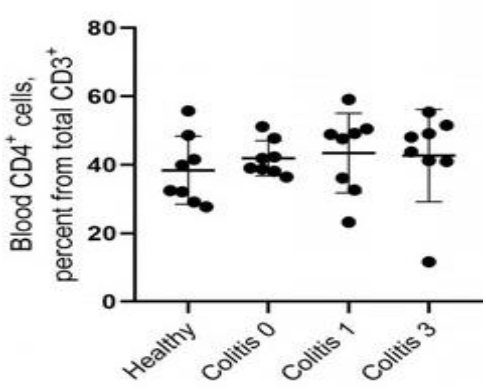


Figure 1. Experimental layout. (a) Sterile donor stool filtrates were prepared, stored at −84 °C, and processed under clean-room conditions one day prior to SFMT. (b) Patient blood and stool samples were collected immediately before SFMT and again at 1 and 3 months post-procedure.

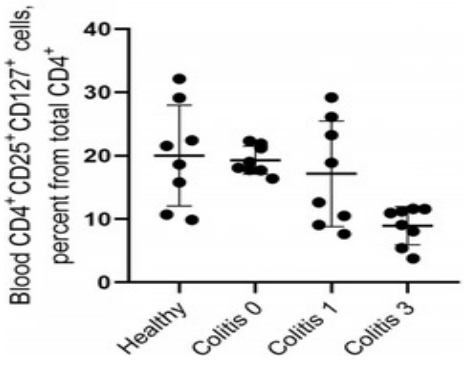
To evaluate the immunological effects, peripheral mononuclear cells collected at each experimental time point were co-cultured with donor and autologous stool filtrates obtained at matching intervals **(Figure 1b)**. Cell activation in vitro was determined by expression of CD25 and CD127, corresponding to IL-2 and IL-7 receptors.

CD25+CD127+ cells represent helper T cells with anti-inflammatory potential, including IL-10–producing subsets [20], while CD25+CD127^low cells correspond to classical regulatory T cells [21].

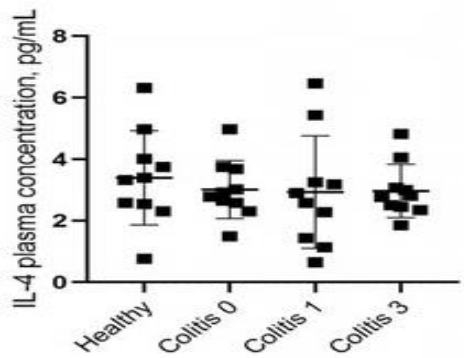
Before analyzing cell responses, it was necessary to measure baseline immune parameters and ensure that stool filtrates did not contain detectable cytokine levels that could artificially affect ex vivo results. Total CD4+ T-cell frequencies in patient blood showed no significant differences over the post-therapy period and did not differ from healthy controls **(Figure 2a)**. Conversely, analysis of CD3+CD4+CD25+CD127^low cells showed a tendency toward reduced regulatory T-cell frequencies at three months after treatment compared with pre-treatment values and with healthy volunteers **(Figure 2b)**. Prior to therapy, Treg levels in patients were comparable to those in healthy controls.



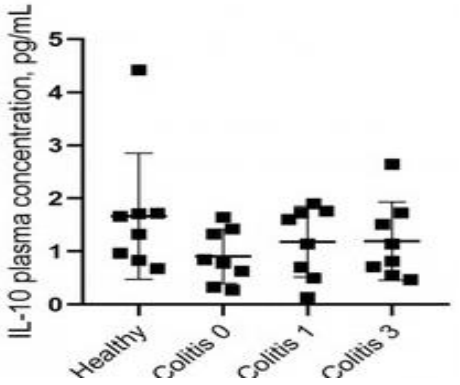
a)



b)



c)

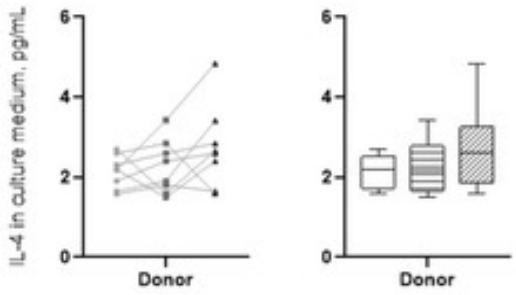


d)

Figure 2. CD4+ T-cell subsets and cytokine profiles in patients and controls. (a) Overall CD4+ content among CD3+ cells. (b) Frequency of CD25+CD127+ activated helper T cells. (c) Plasma IL-4 concentrations. (d) Plasma IL-10 concentrations. Labels: Healthy—healthy controls; Colitis 0—patient blood at baseline; Colitis 1 and Colitis 3—patient blood collected 1 and 3 months post-SFMT.

Influence of sterile fecal filtrates from donors on the helper T cell population in UC patients

We first evaluated how sterile donor stool filtrates affected T-cell activation and cytokine output in vitro. Adding these filtrates to cultures prepared before and after transplantation revealed temporal changes in the prevalence of activated T cells and in cytokine secretion. Cytokine profiling **(Figure 3a and 3b)** showed that donor sterile filtrates did not stimulate IL-4 production—the hallmark cytokine of Th2 responses—in patient cell cultures. In contrast, IL-10 release was markedly elevated in samples obtained three months after therapy. A modest upward trend was also observed at one month, although it appeared only in some individuals. Notably, cells collected before SFMT did not produce IL-10 when exposed to donor sterile filtrates.



a)

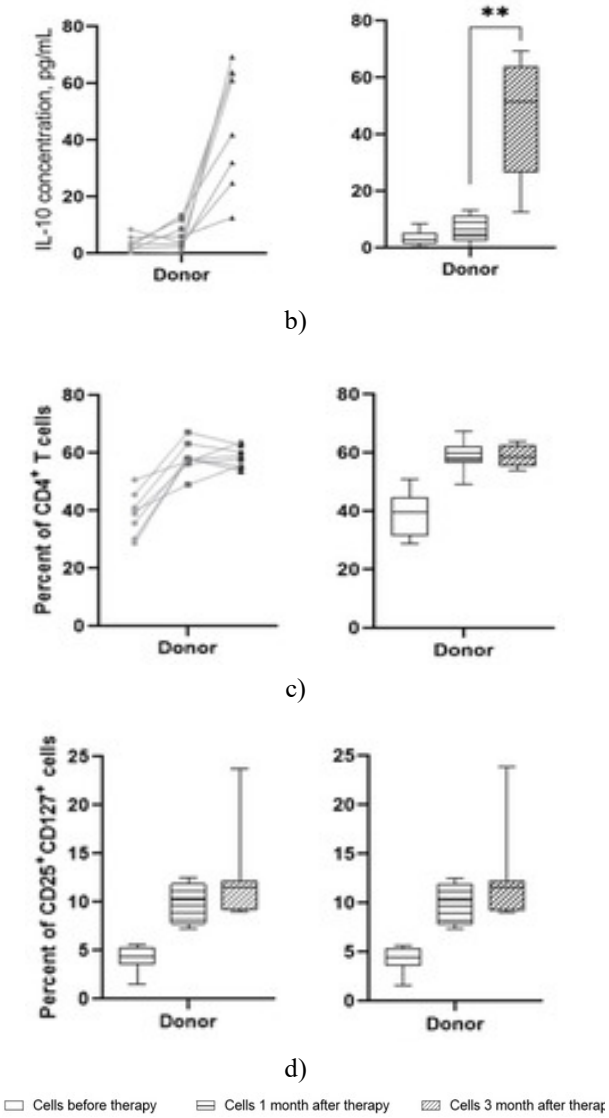
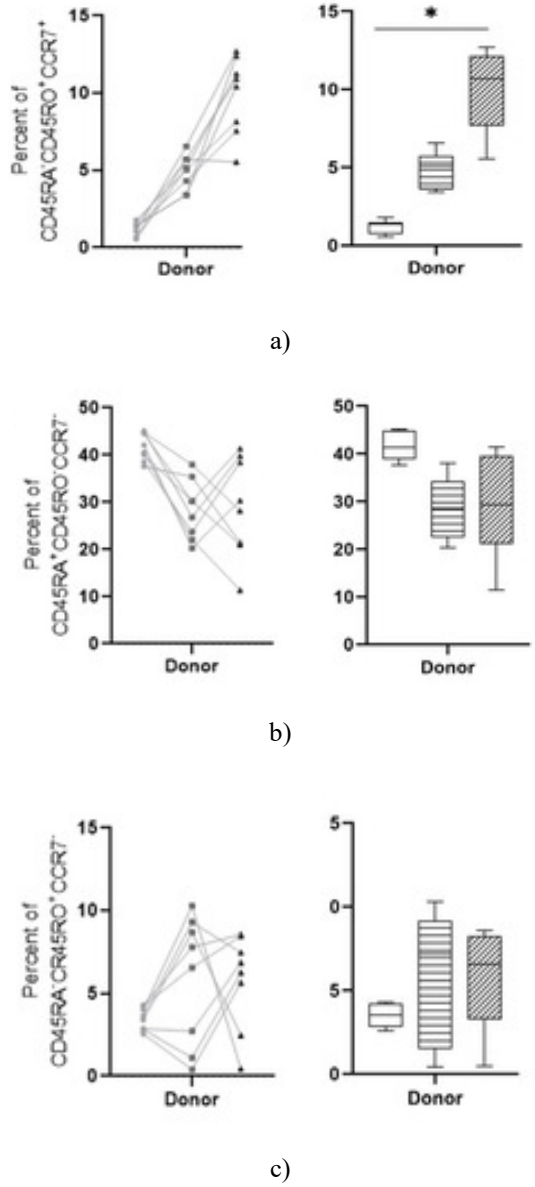


Figure 3. Influence of sterile fecal filtrates from donors on patients' cells

Cells were exposed to GM-CSF and IL-2 for two days. Each parameter is presented both as paired plots (dotted lines) and as median summaries to show within-subject variation and overall group trends. (a) IL-4 concentration in culture supernatants; (b) IL-10 concentration in culture supernatants; (c) proportion of CD4+ T cells among viable cells after two days of ex vivo activation; (d) frequency of CD4+CD25+CD127+ cells capable of producing effector cytokines. ** indicates $p < 0.001$.

Assessment of CD4+ cells in culture demonstrated a progressive increase after therapy **(Figure 3c)**. Examination of activation markers CD25 and CD127 revealed a gradual rise in CD25+CD127+ lymphocytes over time **(Figure 3d)**. When this subset was normalized to total CD4+ cells, the differences became less pronounced. Pre-therapy cells showed weaker responses to donor sterile stool filtrates than cells obtained three months later, suggesting the development of an antigen-directed immune profile. Healthy donor cells reacted to the donor filtrates with increased CD4+ and CD4+CD25+CD127+ frequencies, at levels similar to those of pre-therapy patient cells. These findings imply that donor sterile filtrates—containing minimal antigenic and adjuvant input—support cell survival but do not strongly promote proliferation. No CD25+CD127^low regulatory T cells were detected.

Analysis of activation subpopulations expressing CD45RA, CD45RO, and CCR7 **(Figure 4)** showed that most responding cells were TEMRA cells (CD45RA+CD45RO−CCR7−), the most highly reactive subset. This pattern was seen both when donor filtrates were added to patient cells and when used in cultures of healthy volunteers. However, cells with a central memory phenotype increased only in patient cultures obtained three months after therapy. This effect was not observed in healthy donor cells.



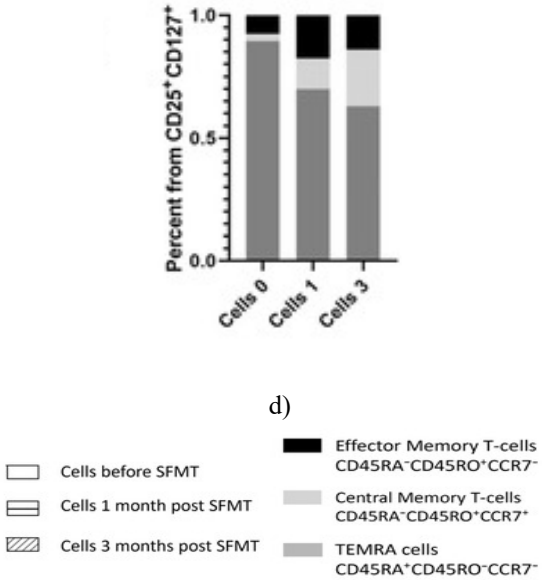
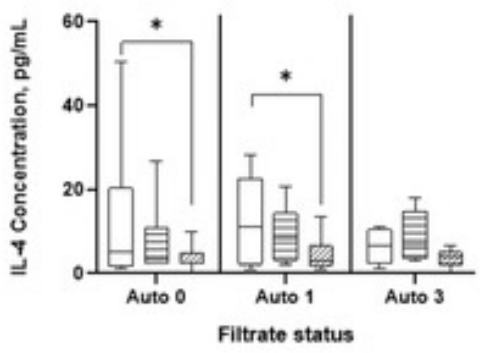


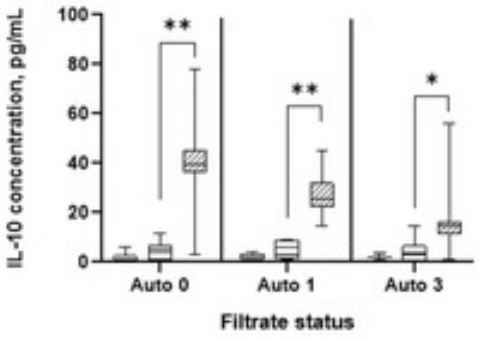
Figure 4. Memory phenotypes of CD25+CD127+ T cells following ex vivo stimulation with donor-derived sterile stool filtrate. Metrics are displayed both as paired profiles (dotted lines) and as median values to illustrate individual trajectories and overall group behavior. (a) Percentage of activated T cells with a central memory phenotype (CD45RA−CD45RO+CCR7+); (b) percentage of TEMRA-type cells; (c) proportion of effector memory cells; (d) relative frequencies of memory subsets in culture. * indicates $p < 0.01$ for effector memory cell percentages.

Autologous sterile fecal filtrates drive changes in IL-4 and IL-10 production

Cytokine secretion by stimulated cells showed clear shifts in IL-4 and IL-10 output **(Figure 5)**. Unlike donor filtrates, autologous samples from baseline (Auto 0) and from one month (Auto 1) could elicit IL-4 production in pre-therapy cells, though this effect was reduced in cells collected at one month. The filtrate obtained three months after therapy (Auto 3) did not substantially trigger IL-4 release. Overall, cells became less responsive to IL-4 induction as the time of sampling increased.



a)



b)

Figure 5. Cytokine dynamics in cells stimulated with autologous filtrates collected at different time points. (a) IL-4 concentration plots; (b) IL-10 concentration plots. * denotes $p < 0.01$, ** $p < 0.001$. Auto 0, Auto 1, and Auto 3 refer to autologous stool samples from baseline, 1 month, and 3 months post-therapy. In contrast, IL-10 production followed the pattern observed with donor filtrates: cells collected at baseline and at one month showed minimal IL-10 release, whereas cells obtained at three months produced markedly higher levels. At the same time, IL-10 production gradually declined when comparing cells isolated at earlier versus later time points. The IL-10/IL-4 ratio was significantly elevated in all groups, although its magnitude varied depending on the filtrate used. Since cytokine release was measured in bulk cultures, the study design did not permit identification of specific cellular sources for IL-4 or IL-10.

Autologous sterile fecal filtrates influence the helper T cell population

To explore how the immunomodulatory properties of patient-derived filtrates changed after SFMT, autologous stool filtrates were added to peripheral mononuclear cell cultures. Filtrates from each study stage were tested on cells from the corresponding time point as well as on cells collected later in the study **(Figure 1b)**. If SFMT altered the microbiota sufficiently to change antigenic composition, subsequent filtrates would be expected to modify activation patterns differently.

CD4+ T-cell frequencies of the cultured cells increased primarily based on the age of the cells themselves, not the age of the filtrate added. ANOVA indicated a strong time effect for the cells ($F = 67.01$, $p < 0.0001$), while the filtrate time factor showed no significant contribution ($F = 1.43$, $p = 0.27$). This pattern is apparent in both paired analyses of cellular shifts **(Figure 6b)** and in overall CD4+ dynamics **(Figure 6a)**.

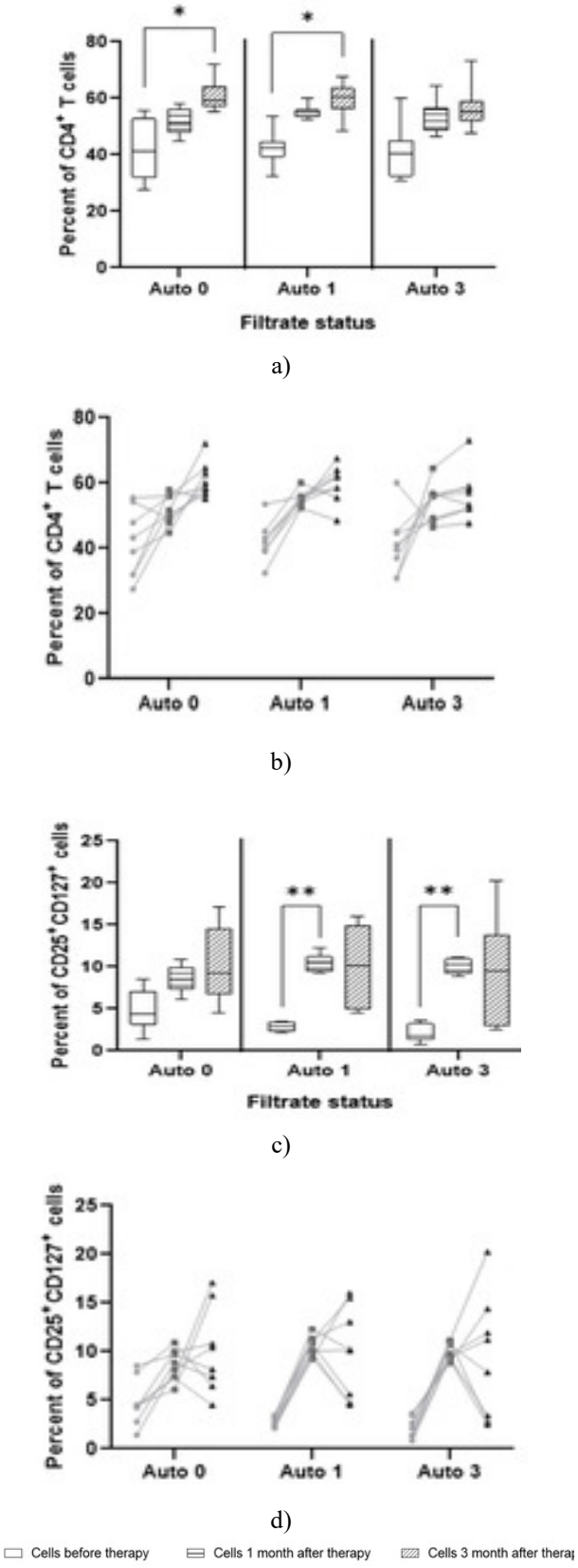
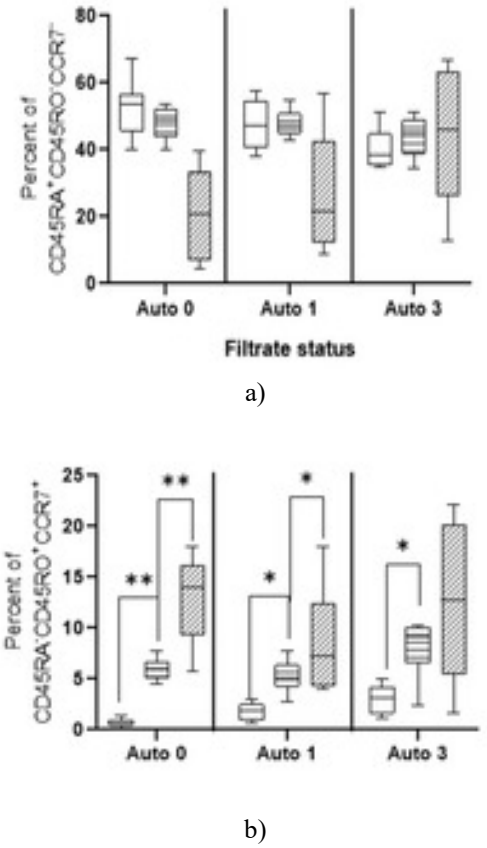


Figure 6. Changes in patient cells stimulated with autologous filtrates over time

All results are presented both as pairwise plots (dotted lines) to show individual patient trends and as median-based plots to illustrate group-level patterns. (a) Percentage of CD4+ T cells in culture, analyzed according to the collection time of the cells or the applied sterile stool filtrate. (b) Same data shown pairwise to highlight patient-specific patterns. (c) Activation status of CD4+ cells measured by CD25 and CD127 surface markers. (d) Pairwise view of activation for each patient. ** denotes $p < 0.001$, * denotes $p < 0.01$.

The frequency of CD25+CD127+ cells **(Figure 6c)** did not strongly depend on which filtrate was applied (ANOVA, $F = 1.2$, $p = 0.31$). Nevertheless, when cultures were stimulated with autologous filtrates from months one and three, clear differences were observed between PBMCs collected at the same time points. Cells from the three-month collection also showed considerable variability in the CD25+CD127+ population, independent of the stimulation source.

Examining memory-associated markers revealed the following trends. As observed with donor filtrates, autologous filtrates led to a predominance of TEMRA cells, although this subset decreased in the three-month samples. Stimulation with Auto 3 filtrates caused a significant rise in TEMRA cells compared to pre-therapy filtrates (Kruskal–Wallis, $p < 0.001$). Conversely, Auto 0 and Auto 1 filtrates resulted in lower TEMRA cell percentages in three-month cells than in cells from earlier time points. Two-way ANOVA indicated that these effects depended both on the timing of cell collection ($F = 20.44$, $p < 0.001$) and the filtrate used for stimulation ($F = 6.49$, $p < 0.001$).



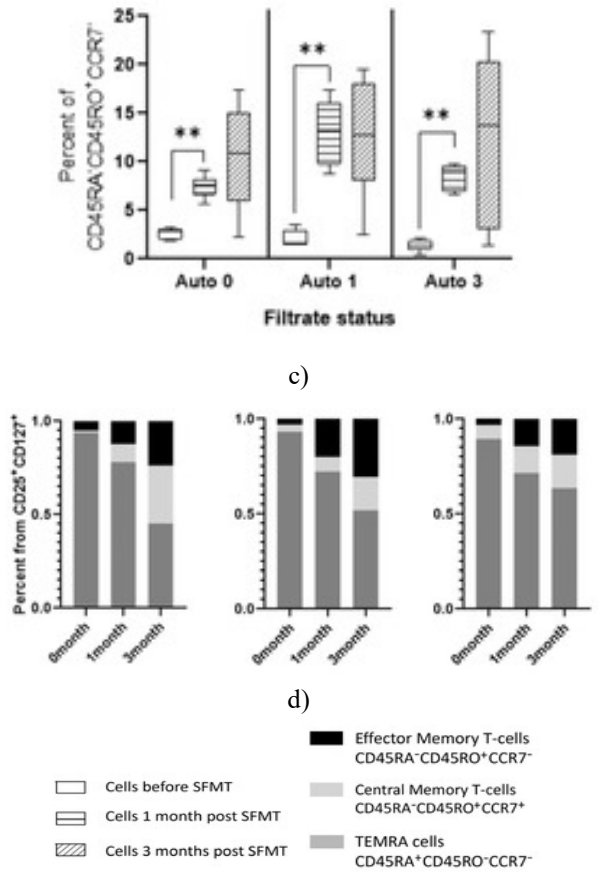


Figure 7. Memory subsets of CD25+CD127+ T cells under ex vivo stimulation with autologous filtrates
Metrics are displayed as paired and median plots to show both intra-individual variation and overall trends. (a) Central memory T cells (CD45RA−CD45RO+CCR7+); (b) TEMRA cells; (c) Effector memory cells; (d) Relative distribution of memory phenotypes. Auto 0, Auto 1, and Auto 3 correspond to autologous stool filtrates collected at baseline, one month, and three months. ** indicates $p < 0.001$; * indicates $p < 0.01$.

Central and effector memory cell proportions were only mildly affected by filtrate type (ANOVA: $F = 4.89$, $p = 0.063$ and $F = 4.03$, $p = 0.009$), whereas the timing of cell collection had a strong influence ($F = 39.4$ and $F = 71.17$, $p < 0.0001$). In particular, cells collected at three months exhibited substantial variability in memory subset composition.

This behavior resembles the patterns seen when donor filtrates were used: depending on the time of cell collection, TEMRA cells were gradually replaced by memory phenotypes. With autologous filtrates (Auto 0 and Auto 1), the shift toward memory populations was more pronounced.

Sterile stool filtrates from patients, both before and after SFMT, contain components that promote the generation of CD25+CD127+ activated T cells. Prior to therapy, these cells predominantly secrete IL-4, whereas cells obtained after therapy—particularly at the three-month mark—tend to produce IL-10. IL-10 secretion is most robust when cells are stimulated with donor filtrates or pre-therapy patient filtrates. Filtrates obtained three months post-therapy do not significantly induce IL-4 and stimulate IL-10 to a lesser degree. Nevertheless, all patient-derived sterile filtrates were capable of inducing IL-10 production in post-SFMT cells.

From these observations, a conceptual model can be proposed to describe how SFMT affects immune cell status. Model 1 suggests that bacteriophages introduced via donor filtrates modulate the gut microbiome, reducing the abundance of pro-inflammatory bacteria. Model 2 posits that donor filtrates primarily alter microbial metabolites, which indirectly modify the composition of intestinal bacteria. In both scenarios, the active factors likely have both antigenic and adjuvant properties. In the first model, therapeutic benefit arises from lowering bacteria that drive persistent pro-inflammatory signals, whereas in the second model, the same immune-specific cells in the patient's gut respond differently due to metabolite-mediated modulation.

It is also important to consider that donor material interacts with the intestinal epithelium for only 2–3 hours, implying that either the factors are highly potent or short-term effects trigger longer-lasting outcomes. From this perspective, Model 1 appears more plausible. If appropriate host bacteria are present and corresponding bacteriophages exhibit moderate lytic activity, these phages can persist in the gut ecosystem, regulating bacterial populations over time. This mechanism has been previously demonstrated in sterile microbiota transplantation for patients with Clostridioides infections [22], which led to a notable increase in Caudoviricetes-class bacteriophages [22] and a relative increase in Firmicutes versus Bacteroidetes [23].

Although this model can explain improvement in disease severity, it does not fully account for the skewing of helper T-cell responses. Our results indicate that elevated IL-10 production occurs regardless of filtrate type, suggesting that post-therapy timing is more critical than antigenic load.

Previous studies have shown that FMT can shift helper T cells toward regulatory and IL-10-producing phenotypes [18]. In patients with IBD, FMT was associated with increased IL-10 and decreased IL-17 levels in circulation [19], effects initially attributed to successful colonization by healthy donor microbiota and increased production of metabolites that favor regulatory T cells. However, other reports suggest that engraftment is not the key determinant of therapeutic benefit; instead, small molecules produced by donor microbiota, such as SCFAs and bile acids, can independently promote regulatory T-cell differentiation. Yet the effects of these low-molecular-weight compounds

are limited to short-term exposure, which may not produce lasting immunomodulation.

It is possible that a combination of antigen-dependent and antigen-independent factors drives the shift in helper T-cell phenotype. Further research is needed to clarify which bacteriophages influence microbiota composition, which bacterial species impact T-cell polarization, and whether the effects of SFMT are durable or transient. It is also likely that no single, standardized SFMT formulation exists, making the therapy inherently patient-specific. Nonetheless, SFMT represents a promising approach that targets the underlying immunological mechanisms of UC rather than just alleviating symptoms.

Regarding limitations, the small patient cohort is a major constraint due to the experimental nature of the procedure and strict inclusion criteria. Patients showing clinical deterioration or systemic inflammatory signs prior to the planned intervention were excluded. Other limitations include the absence of a healthy volunteer comparison group. Future studies should address these issues by expanding patient numbers, including healthy participants, and broadening the panel of markers used to characterize lymphocyte subsets.

It is proposed that the observed changes in immune response involve both innate and adaptive mechanisms. Donor stool filtrates deliver bioactive molecules, including short-chain fatty acids (SCFAs) and bacterial cell wall fragments. One intriguing possibility is that bacteriophages present in donor filtrates [24] reshape the recipient's intestinal microbiome. In this scenario, the reduction in T-cell pro-inflammatory activity could result from the removal of bacterial strains that serve as antigenic stimuli for the patient. However, this explanation does not account for the persistence of altered T-cell responses observed one and three months post-FMT. An alternative explanation is that bacteriophages act as an initial antigenic trigger, activating T cells via antigen-presenting cells. Once primed, these cells may maintain heightened responsiveness, helping to prolong immune modulation.

Overall, the findings suggest a potential new therapeutic strategy for inflammatory bowel disorders that could be described as gut-targeted immune conditioning. The gradual shift in helper T-cell phenotype over time post-FMT implies that the effect is antigen-specific and evolves progressively.

Materials and Methods

Donors

All stool donors were healthy young adults aged 20–25 years, without chronic illness or recent hospitalizations (minimum of two months). Donors underwent extensive screening, including biochemical and general blood tests, and ELISA assays for Giardia, Toxocara, Opisthorchis, Ascaris, and Trichinella. Serological testing was performed for Treponema pallidum ("Vector-Best" LLC, Novosibirsk, Russia), HIV types 1 and 2 ("Vector-Best" LLC), and hepatitis B and C viruses ("Vector-Best" LLC). Fecal analysis included routine microbiology for Salmonella spp., Shigella spp., enteroinvasive Escherichia coli, and Cryptosporidium spp., as well as microscopic evaluation for helminths and their eggs. Toxins A and B of Clostridioides difficile were detected using a rapid immunochromatographic kit (Toxin A + B (Clostridium difficile) DUO, Vedalab, Alençon, France). DNA from Shigella spp., enteroinvasive E. coli, Salmonella spp., and Campylobacter was assessed via PCR with fluorescence-based hybridization (Amplisence OKI Bacto-Screen-FL, Moscow, Russia). Rotaviruses A, noroviruses I and II, and adenoviruses F were screened using the same approach (Amplisence OKI Viro-Screen-FL, Moscow, Russia). Donors were additionally tested for Helicobacter pylori.

Ethical approval was granted by the Local Ethics Committee of the Autonomous Non-Commercial Organization "Center of New Medical Technologies in Akademgorodok" (Protocol #2, 12 January 2019). Written informed consent was obtained from all donors and patients prior to participation and throughout follow-up.

Patients

Adult participants of any gender with mild UC (UCEIS 2–4) were enrolled at the Center for New Medical Technologies, SB RAS, between December 2021 and January 2023. Eligibility criteria included UC scores of 2–6 confirmed by colonoscopy and clinical evaluation, and an ESR < 30 mm/hour. All patients were screened for Clostridioides difficile and H. pylori, underwent ECG evaluation, and were assessed by a therapist and gastroenterologist; an anesthesiology evaluation was performed prior to colonoscopy. Stool samples collected before treatment were analyzed for the pathogens listed in Section 4.1.

Exclusion criteria were age <18 or >79, pregnancy, confirmed irritable bowel syndrome or Crohn's disease, and presence of C. difficile or H. pylori. SFMT was postponed if patients displayed increased stool frequency, elevated ESR, or laboratory evidence of inflammation (CRP, WBC) on the day of the procedure or early in the follow-up period. Patients were monitored for three months post-procedure, including follow-up blood tests and colonoscopy.

Preparation of sterile fecal filtrate

Donor stool was collected in a controlled, sterile environment specifically set up for sample handling. A portion of each stool sample was allocated for routine laboratory testing, while the remaining material was

subdivided into equal portions exceeding 100 g and stored at −84 °C.

On the day before SFMT, these frozen samples were processed to produce sterile filtrates. All procedures followed the internal standard protocols of the Center for Novel Medicine Technologies and were conducted under aseptic conditions. For preparation, the thawed stool was combined with 300 mL of sterile 0.9% NaCl solution and homogenized using a mechanical tissue grinder. The homogenate was sequentially passed through sterile filter cascades with pore sizes of 0.45 μm and 0.22 μm, and the filtrate was collected into GMP-compliant sterile disposable containers. Prepared filtrates were stored at +4 °C overnight. Two hours prior to the transplantation, the material was transferred into an aseptic container to equilibrate the temperature. During fibrocolonoscopy, the filtrates were administered directly into the patient's colon.

Collection of patient samples

Patient blood and stool were collected at three time points: prior to SFMT, and at one and three months after the procedure. Blood samples were drawn in the morning and transported to the laboratory in K3 EDTA tubes. Stool specimens were stored at −20 °C until further analysis.

Isolation of PBMCs and plasma from blood

PBMC isolation was initiated within two hours of blood collection. First, samples were centrifuged at 300×g for 10 minutes, and 1.5–2 mL of the bottom fraction was collected. The remaining blood was diluted 1:2 with calcium-free phosphate-buffered saline and gently layered over 15 mL conical tubes containing Ficoll Histopaque-1077 at a 3:1 blood-to-Ficoll ratio. Tubes were centrifuged at 400×g for 30 minutes using a swinging-bucket rotor with slow acceleration and deceleration settings. The upper plasma layer was removed, and the PBMC layer at the interphase was harvested. Cells were washed three times with phosphate-buffered saline and counted. A fraction was immediately stained with fluorescent antibodies for immunophenotypic analysis.

Cryopreservation of PBMCs

Cells were resuspended in freezing medium composed of fetal bovine serum and DMSO (9:1) at a concentration of 5×10^6 cells/mL. Freezing was performed in two steps: first, using isopropanol-based freezing containers at −70 °C, followed by transfer to liquid nitrogen vapor for long-term storage. Plasma samples were stored at −70 °C.

PBMC culture and stimulation

Isolated PBMCs were cultured in RPMI-1640 medium supplemented with heat-inactivated calf serum and 4 mM glutamine. On the day of the experiment, thawed cells were washed to remove cryoprotectant and resuspended in fresh medium containing GM-CSF at 40 ng/mL to activate monocytes and support antigen presentation. Cells were seeded at 2×10^5 per well in 100 μL medium in 96-well plates and incubated with either donor-derived or the patient's own sterile filtrates. Cultures were maintained for four days. During this period, the formation of cellular clusters was monitored and photographed. At the end of cultivation, supernatants were collected for enzyme immunoassays, and cells were stained with surface markers to assess phenotypic changes related to activation.

Flow cytometric analysis of fresh and cultured PBMCs

Peripheral blood mononuclear cells were labeled with fluorescent antibodies targeting surface markers to identify lymphocyte subpopulations (BD Biosciences, Franklin Lakes, NJ, USA). The antibody panel included: CD3-(FITC), CD4-(BV510), CD25-(PE), CD197-(BV421), CD45RA-(PerCP), and CD45RO-(APC-Cy7). This set was designed to detect regulatory T cells and characterize them with respect to their memory and effector properties.

Cells were incubated with the antibodies in phosphate-buffered saline containing 0.5% bovine serum albumin for 1 hour at +4 °C. Following staining, cells were fixed with 4% formalin for 15 minutes, then residual formalin was neutralized using 0.15 M glycine solution. Flow cytometric acquisition was performed on a Novocyte 3000 instrument (ACEA Biosciences Inc., San Diego, CA, USA).

Cytokine measurements in plasma and sterile stool filtrates

Cytokine concentrations in plasma were determined using solid-phase ELISA kits (Vector-Best) according to manufacturer instructions. Plasma samples were thawed at +4 °C the day before analysis. For sterile stool filtrates, the amount of sample added to ELISA wells was standardized relative to the weight of the original stool material from which the filtrate was obtained.

Data processing and statistical analysis

Flow cytometry data were analyzed using NovoExpress v.1.6.1. Statistical evaluations were conducted with GraphPad Prism v.8 and Python 3.7. Paired, time-dependent comparisons were performed using paired t-tests followed by Tukey post-hoc tests. For comparing multiple stool filtrate groups, Kruskal–Wallis tests with Dunn's post-hoc corrections were applied.

Conclusion

This pilot study examined T-cell responses to sterile fecal transplantation in patients with ulcerative colitis. The

primary goal was to assess how T-cell activity might shift in response to SFMT and identify patterns potentially linked to therapeutic benefit. The study was based on the hypothesis that sterile fecal filtrates, containing antigenic or adjuvant-like components but lacking live microorganisms, could induce a transition of T-cells from a pro-inflammatory to an anti-inflammatory or anergic state.

Results demonstrated that peripheral T-cells altered their response profiles following SFMT. Specifically, ex vivo stimulation with sterile filtrates resulted in a transition from dominant IL-4 production to increased IL-10 output. Cells exhibiting the CD3+CD4+CD25+CD127+ phenotype showed the greatest survival during ex vivo culture, and their memory phenotypes were predominantly TEMRA and effector memory cells.

Although further studies are needed, particularly to identify the active components within the sterile filtrates, this work provides the first direct evidence that sterile fecal components can modulate T-cell states in UC patients. These findings may form the foundation for developing a new class of anti-inflammatory therapies applicable not only to ulcerative colitis but potentially to other inflammatory bowel conditions.

Acknowledgments: None

Conflict of interest: None

Financial support: All immunological investigations were funded by the Russian Science Foundation, Project No. 21-14-00360. Blood and stool sampling was supported by the Ministry of Education and Science, Project No. 121031300043-8.

Ethics statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Local Ethics Committee of the Center for Personalized Medicine, Novosibirsk (protocol #2, date of approval: 12 January 2019) including written consent from patients and healthy volunteers to present their blood and fecal samples for scientific purposes (according to the guidelines of the Helsinki Ethics Committee).

Informed consent was obtained from all healthy volunteers and patients involved in the study.

References

1. AlAmeel T, AlMutairdi A, Al-Bawardy B. Emerging therapies for ulcerative colitis: updates from recent clinical trials. Clin Exp Gastroenterol. 2023;16:147–67.
2. Ahluwalia B, Moraes L, Magnusson MK, Öhman L. Immunopathogenesis of inflammatory bowel disease and mechanisms of biological therapies. Scand J Gastroenterol. 2018;53(4):379–89.
3. Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schäffer AA, Noyan F, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N Engl J Med. 2009;361(21):2033–45.
4. Shah N, Kammermeier J, Elawad M, Glocker EO. Interleukin-10 and interleukin-10 receptor defects in inflammatory bowel disease. Curr Allergy Asthma Rep. 2012;12(4):373–9.
5. Negi S, Das DK, Pahari S, Nadeem S, Agrewala JN. Potential role of gut microbiota in induction and regulation of innate immune memory. Front Immunol. 2019;10:2441.
6. Shi N, Li N, Duan X, Niu H. Interaction between the gut microbiome and mucosal immune system. Mil Med Res. 2017;4(1):14.
7. Basha OM, Hafez RA, Salem SM, Anis RH, Hanafy AS. Impact of gut microbiome alteration in ulcerative colitis patients on disease severity and outcome. Clin Exp Med. 2023;23(6):1763–72.
8. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A. 2010;107(27):12204–9.
9. Liu X, Zou Q, Zeng B, Fang Y, Wei H. Analysis of fecal Lactobacillus community structure in patients with early rheumatoid arthritis. Curr Microbiol. 2013;67(2):170–6.
10. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, et al. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. eLife. 2013;2:e01202.
11. Wu R, Xiong R, Li Y, Chen J, Yan R. Gut microbiome, metabolome, and host immunity in inflammatory bowel disease and fecal microbiota transplantation. J Autoimmun. 2023;141:103062.
12. Chu ND, Crothers JW, Nguyen LTT, Kearney SM, Smith MB, Kassam Z, et al. Dynamic colonization of microbes after fecal microbiota transplantation for inflammatory bowel disease. mBio. 2021;12(3):e00975-21.
13. Marrs T, Walter J. Is faecal microbiota transplantation a safe and efficient treatment option for gut dysbiosis? Allergy. 2021;76(8):2312–7.
14. Jaramillo AP, Awosusi BL, Ayyub J, Dabhi KN, Gohil NV, Tanveer N, et al. Effectiveness of fecal microbiota transplantation in inflammatory bowel disease: a systematic review. Cureus. 2023;15(4):e42120.
15. Gogokhia L, Round JL. Immune–bacteriophage interactions in inflammatory bowel diseases. Curr Opin Virol. 2021;49:30–5.

16. Federici S, Kviatcovsky D, Valdés-Mas R, Elinav E. Microbiome–phage interactions in inflammatory bowel disease. Clin Microbiol Infect. 2023;29(6):682–8.
17. Draper LA, Ryan FJ, Smith MK, Jalanka J, Mattila E, Arkkila PA, et al. Long-term colonisation with donor bacteriophages following faecal microbial transplantation. Microbiome. 2018;6(1):220.
18. Burrello C, Garavaglia F, Cribiù FM, Ercoli G, Lopez G, Troisi J, et al. Therapeutic faecal microbiota transplantation controls intestinal inflammation through IL-10 secretion. Nat Commun. 2018;9(1):5184.
19. Quraishi MN, Shaheen W, Oo YH, Iqbal TH. Immunological mechanisms underpinning faecal microbiota transplantation in inflammatory bowel disease. Clin Exp Immunol. 2019;199(1):24–38.
20. Narsale A, Lam B, Moya R, Lu T, Mandelli A, Gotuzzo I, et al. CD4+CD25+CD127hi cell frequency predicts disease progression in type 1 diabetes. JCI Insight. 2021;6(4):e136114.
21. Gołąb K, Krzystyniak A, Marek-Trzonkowska N, Misawa R, Wang LJ, Wang X, et al. Impact of culture medium on CD4+CD25highCD127lo/neg Treg expansion. Int Immunopharmacol. 2013;16(3):358–63.
22. Zuo T, Wong SH, Lam K, Lui R, Cheung K, Tang W, et al. Bacteriophage transfer during faecal microbiota transplantation is associated with treatment outcome. Gut. 2018;67(4):634–43.
23. Boicean A, Birlutiu V, Ichim C, Anderco P, Birsan S. Fecal microbiota transplantation in inflammatory bowel disease. Biomedicines. 2023;11(4):1016.
24. Liu Q, Xu Z, Dai M, Su Q, Leung Chan FK, Ng SC. Faecal microbiota transplantation and the role of bacteriophages. Clin Microbiol Infect. 2023;29(6):689–94.