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Basic Molecular Biology, Metabolic and Immunological Mechanisms of Fecal Microbiota Transplantation

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Aim: demonstration of basic molecular biological, metabolic and immunological effects of fecal microbiota transplantation (FMT), on the example of a rare case of acute graft-versus-host disease (GVHD) with intestinal damage in a patient after allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Materials and methods. To monitor the basic effects of FMT, we performed targeted DNA sequencing of *16S* rRNA gene (V3–V4) using MiSeq platform as well as multiplex real-time PCR, MS/gas chromatography technique, immunophenotyping of blood lymphocytes, histological and immunohistochemical techniques.
Clinical case. A 40-year-old female patient diagnosed with myelodysplastic syndrome, with a history of two unsuccessful allo-HSCTs due to graft failure, underwent the third haploidentical HSCT (haplo-HSCT) from her father as 'salvage' therapy. Due to early viral/bacterial colitis post-transplant associated with a multidrug-resistant strain of *K. pneumoniae* and herpes virus type 6, FMT was performed on days 46 and 47 after allo-HSCT. Complete resolution of the enteropathy symptoms was noted following FMT. However, immunosuppressive therapy was canceled on D+106 after haplo-HSCT due to the detection of minimal residual disease causing development of the 'overlap'-type GVHD with damage skin lesions grade 4, and intestinal mucous membranes grade 3. This complication required resumption and subsequent intensification of immunosuppressive therapy with complete resolution of GVHD symptoms.
Following FMT treatment, the patient showed complete resolution of clinical colitis symptoms. According to results of *16S* rRNA sequencing, the species-specific diversity of fecal microbiota increased significantly, along with decreased relative contents of opportunistic bacteria (*Klebsiella, Enterococcus, Streptococcus* genera). A significant growth was revealed for commensal *Bacteroidota*, and re-emergence of *Faecalibacterium, Blautia, Roseburia*. Acute gastrointestinal GVHD promoted by tacrolimus withdrawal was associated with repeated depletion of intestinal microbiota. Upon resolution of GVHD and resumed immunosuppression, increased microbiota diversity (Shannon index) was again recorded, and the parameters of patient's fecal microbiota reached the donor values. The microbiota shifts at all clinical stages (before and after FMT, at the peak of acute intestinal GVHD and intensive immunosuppressive therapy) showed some relations with metabolism of bile and fatty acids in blood plasma and immune parameters.
Conclusions. FMT may be a component of complex therapy aimed at early reconstitution of immune system and organic acid metabolism in patients after allo-HSCT. The composition of fecal microbiota, metabolic profile and spectrum of lymphocyte subpopulations may be markers for monitoring complex rehabilitation after allo-HSCT.
Keywords: fecal microbiota transplantation, graft-versus-host disease, allogeneic hematopoietic stem cell transplantation, metabolome, bile acids, lymphocyte subpopulation
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Conflict of interests: Evgenyi A. Bakin performed the study at the R. Gorbacheva Memorial Research Institute of Pediatric Oncology, Hematology and Transplantology, Pavlov First Saint Petersburg State Medical University (Saint Petersburg, Russia). Currently, Evgenyi A. Bakin is a staff member at the CytoReason Compamy (Tel-Aviv, Israel). Natalia D. Venclovaite performed the study at the R. Gorbacheva Memorial Research Institute of Pediatric Oncology,

Hematology and Transplantology, Pavlov First Saint Petersburg State Medical University (Saint Petersburg, Russia). Currently Natalia D. Venctslovayte is a staff member of the German Medical Center (Russia). Other authors did not declare any conflicts of interest.
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Основные молекулярно-биологические, метаболические и иммунологические механизмы трансплантации фекальной микробиоты

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Цель представления клинического наблюдения: продемонстрировать основные молекулярно-биологические, метаболические и иммунологические эффекты трансплантации фекальной микробиоты (ТФМ) на примере редкого случая развития острой реакции «трансплантат против хозяина» с поражением кишечника (РТПХ ЖКТ) у пациентки после проведения аллогенной трансплантации гемопоэтических стволовых клеток (алло-ТГСК).

Материалы и методы. Для оценки основных, известных механизмов ТФМ было использовано таргетное секвенирование фрагментов V3–V4 участка гена *16S* рРНК на платформе Miseq (16s секвенирования), мультиплексная полимеразная цепная реакция в режиме реального времени, хромато-масс-спектрометрия, иммунофенотипирование субпопуляций лимфоцитов, гистологический и иммуногистохимический методы исследования.

Клинический случай. Пациентке 40 лет с диагнозом «миелодиспластический синдром», анамнезом двух неуспешных алло-ТГСК в связи с неприживлением трансплантата в качестве «терапии спасения» была выполнена третья гаплоидентичная ТГСК от отца. В связи с развитием в раннем посттрансплантационном периоде вирусно-бактериального колита (ассоциированного с полирезистентным штаммом *K. pneumoniae* и вирусом герпеса 6-го типа) на 46–47-е сутки после алло-ТГСК была выполнена ТФМ. После проведенной процедуры отмечалось полное разрешение симптомов энтеропатии. Однако на Д+106 после гаплоидентичной ТГСК в связи с детекцией минимальной остаточной болезни была отменена иммуносупрессивная терапия, что привело к развитию РТПХ по типу overlap-синдрома с поражением кожи 4-й степени, слизистых оболочек кишечника 3-й степени. Это потребовало возобновления и в последующем усиления иммуносупрессивной терапии, на фоне чего отмечалось полное разрешение симптомов РТПХ.

После выполнения ТФМ у пациентки отмечено полное разрешение клинических симптомов вирусно-бактериального колита. По результатам 16s секвенирования, значимо возросло видовое разнообразие фекальной микробиоты, выявлено уменьшение относительного количества условно-патогенных бактерий (род *Klebsiella, Enterococcus, Streptococcus*), уверенный рост комменсальных микроорганизмов: *Bacteroides*, появление родов *Faecalibacterium, Blautia, Rozeburia*. Развитие острой РТПХ ЖКТ, спровоцированной отменой такролимуса, способствовало повторному эпизоду обеднения микробиоты кишечника. По мере разрешения симптомов РТПХ на фоне возобновления иммуносупрессии был вновь зафиксирован рост индекса Шеннона, а состав фекальной микробиоты пациентки приблизился к таковому у донора. Состав микробиоты на всех клинических этапах — до и после ТФМ, в период максимальной манифестации симптомов острой РТПХ кишечника, усиления иммуносупрессивной терапии — оказывал влияние на метаболизм желчных, жирных кислот плазмы крови и на показатели иммунной системы.

Выводы. ТФМ может быть частью терапии, направленной на раннюю реконституцию иммунной системы и обмена органических кислот, у пациентов после алло-ТГСК. Состав фекальной микробиоты, метаболический профиль и спектр субпопуляции лимфоцитов могут быть маркерами контроля комплексной реабилитации после алло-ТГСК.

Ключевые слова: трансплантация фекальной микробиоты, реакция «трансплантат против хозяина», аллогенная трансплантация гемопоэтических стволовых клеток, метаболом, желчные кислоты, субпопуляция лимфоцитов

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the most effective treatment for most hematopoietic malignancies [1–3]. A graft-versus-tumor response is among the main effects of allo-HSCT. It is caused by recognition of histocompatibility antigens on the recipient's malignant cells followed by their destruction by T lymphocytes of donor origin [1, 4]. However, activated T-lymphocytes are able to recognize the same antigens on normal cells of the recipient tissue, leading in turn to the development of a graft-versus-host disease (GVHD) [1].

Relapses of the underlying disease occur in approximately 25 % of patients after allo-HSCT [4]. Predictors of post-transplant relapse may include detection of minimal residual disease and/or reduction in donor chimerism, which often requires reduction or even complete discontinuation of immunosuppressive therapy. It is known that such tactics can induce the development of GVHD [4, 5].

Involvement of gastrointestinal tract is the most severe and difficult-to-cure manifestations of acute GVHD [6]. To date, a correlation is shown between altered composition of fecal microbiota (FM) and GVHD development [7]. Allo-HSCT and accompanying therapy (conditioning regimen, antibacterial therapy and low-microbial diet) have a significant negative impact on the composition and species diversity of FM [8]. Reduced contents of the main phyla of commensal bacteria, such as *Bacillota, Bacteroidota, Actinomycetota*, leads to excessive colonization of intestinal tract with opportunistic pathogens such as Proteobacterota (*Gammaproteobacteria*), certain *Bacillota* genera (*Enterococcus* spp., *Staphylococcus* spp., etc.). These bacteria induce production of pro-inflammatory cytokines (IL-1, IL-6), leading to activation of T lymphocytes and development of acute GVHD of the gastrointestinal tract [3, 6, 7, 9].

Over recent years, fecal microbiota transplantation (FMT) was established as an effective strategy to correct the intestinal biocenosis in many gastrointestinal diseases and an approach to biological decolonization of antibiotic-resistant strains [10–12], with proven effectiveness and safety in the cohorts of immunocompromised patients [13], including those with acute gastrointestinal GVHD and overlap syndrome [2, 14].

The results of recent studies have demonstrated the role of FM in regulation of gene expression, modulation of immune and metabolic processes in the whole body [15–18]. Commensal bacteria from gut microbiota produce short-chain fatty acids, bile acids, amino acids, which interact with specific receptors, such as TLR, NLR, G-protein receptors (GPR43, 41, 109A), FXR, which are involved in regulation of immunity and metabolism in the human host [15].

The presented clinical case demonstrates for the first time distinct changes in gut microbiota composition, certain dynamics of cellular and humoral immune response, shifts in bile and unsaturated fatty contents at all stages of treatment for acute gastrointestinal GVHD in a patient after allo-HSCT.

Materials and methods

Fecal samples were collected at 15 time-points: before FMT, on days +1 after allo-HSCT (D+1), days +3, +8, +16, +30, +45, +60, +70, +75, +105, +120, +140, +200, and +260 after FMT. Bacterial composition and biodiversity of FM were assessed by means of targeted DNA sequencing of *16S* rRNA gene (V3–V4 fragments) performed on the MiSeq platform ("Illumina", USA) in accordance with the standard protocol 16S Metagenomic Sequencing Library Preparation ("Illumina", USA). The V3–V4 fragment amplification was carried out using Phusion High-Fidelity DNA Polymerase ("Thermo Fischer Scientific", USA) with proofreading activity. Nextera XT Index Kit ("Illumina", USA) was used to multiplex DNA libraries. Sequencing was performed using 600-cycle MiSeq Reagent Kit v3 ("Illumina", USA) to obtain paired reads of 300 nucleotides long. Quality control of NGS performed for each sample included the following parameters: at least 50,000 sequence reads per sample; the optimal number of high-quality reads with a Q30 value of more than 60 %; absence of chimeric sequences and overrepresented sequences.

To evaluate total bacterial mass in the FM samples, the real-time multiplex PCR technique was used with a commercial test system "Kolonoflor-16" (OOO "Alfalab", Russia) [19].

Chromato-mass spectrometry (MS) was used to determine concentrations of bile acids in blood plasma. The MS studies were performed at the "Agilent 1200" high-performance liquid chromatograph with an "Agilent 6460" triple quadrupole ("Agilent Technologies", USA). Polyunsaturated fatty acids (PUFAs) have been quantified with "Agilent 7890" gas chromatograph with a mass-selective detector ("Agilent Technologies", USA).

Immunophenotyping of lymphocyte subpopulations was performed on a "Cytomics FC500" flow cytometer ("Beckman Coulter", USA).

Histological and immunohistochemical studies of sections, embedded in paraffin tissue samples, were carried out using standard techniques. Routine hematoxylin and eosin staining of sections and immunohistochemical determination of CD3 expression in the tissue were used.

This study was approved by the local ethics committee of the Pavlov First Saint Petersburg State Medical University Protocol No. 192 of January 30, 2017.

Clinical case

Patient K., 40 -years-old woman, was diagnosed in 2007 with acquired aplastic anemia and paroxysmal nocturnal hemoglobinuria complicated with severe hemolytic crises. Therapy with cyclosporine and eculizumab was carried out for 10 months resulting in stable clinical and laboratory remission which lasted 10 years.

In 2017, blood counts revealed two-lineage cytopenia (grade 2 thrombocytopenia, grade 1 anemia). Trephine biopsy revealed myelodysplastic syndrome, refractory anemia with excess blast-II (RAEB II) was diagnosed.

Two courses of therapy with hypomethylating agents resulted into stabilization of the disease.

In April 2018, the patient underwent allo-HSCT from a completely HLA-matched donor found in the international registry. Complete clinical and hematological remission was achieved, with restoration of donor-type hematopoiesis. However, a decrease in donor chimerism to 80–89 % was observed at early terms after allo-HSCT thus requiring early withdrawal of immunosuppressive therapy (since D+64), administration of hypomethylating agents, and the infusion of donor lymphocytes. However, at the control restaging (D+100), monosomy of chromosome 7 and a decrease in donor chimerism to 20–30 % were revealed, and graft rejection was detected due to relapse of the disease. Therefore, a decision was made to perform a repeated HSCT from the same donor. However, after second transplant (August 2018), hematopoiesis was restored with recipient cells (donor cell ratio < 3 %, monosomy of chromosome 7 in 100 % of metaphases) thus confirming primary non-engraftment.

Due to recovery of the own patient's hematopoiesis, absence of "graft versus leukemia" effect, and persistence of primary malignant disease, a decision was made to perform haploidentical HSCT from her father as a part of "salvage therapy" strategy.

After the third haploidentical HSCT, the post-transplant period proceeded as follows:

– ***D+3 to D+7***, after allo-HSCT, – grade 3 cytokine release syndrome has been developed (febrile fever, cytolysis, maculopapular skin rash). Therefore, therapy with ruxolitinib was started at a dose of 10 mg/day followed by resolution of skin rashes; ruxolitinib was discontinued on D+29.

– ***D+15*** – engraftment of the transplant, clinical and hematological remission of underlying disease, donor chimerism of 95–97 % were registered.

– ***D+25*** – development of diarrhea up to 7 times daily, 1300 mL/day, without pathological admixtures. Stool bacteriology showed high levels (10^8 CFU/mL) of pathogenic *K. pneumoniae* which was resistant to most groups of antibacterial drugs (penicillins, cephalosporins, fluoroquinolones, carbapenems). Therefore, a decision was made to carry out pharmacological decontamination with oral amikacin

(1000 mg/day) added to the therapy protocol. Intact mucosal surface was seen at fibrocolonoscopy. Histomorphological study of intestinal walls revealed mild stromal fibrosis and minimal reactive changes of glandular epithelium (Fig. 1, a). DNA PCR assay of colonic mucosa lysate showed human herpes virus type 6 (HHV6). Therefore, viral colitis was diagnosed, and ganciclovir (10 mg/kg/day) was added to therapy.

– ***D+31*** – positive changes were observed in the course of antibacterial and antiviral therapy, i.e., decreased frequency of stools (3 times and 300 mL/day). However, despite the therapy, bacteriological tests of feces, urine and throat swabs still revealed the multi-resistant strain of *K. pneumoniae*. In view of persisting enteropathy, biological decolonization of *K. pneumoniae* was decided by means of fecal microbiota transplantation (FMT).

– ***D+46 and D+47*** after haploidentical HSCT – FMT was performed from a healthy unrelated donor. The patient took 15 capsules of frozen FM daily on two subsequent days. The total mass of fecal transplant was 22 g. Before signing the informed consent, all the benefits, disadvantages and side effects of the FMT method were explained to the patient. The protocols for examining the fecal transplant donor, preparing capsules with frozen FM, and the protocol for taking capsules with FM were described in previous publications [10, 14].

– ***D+49–57 (D+3–11 after FMT)*** – positive dynamics manifested as a decreased stool frequency and volume. On D+8 after FMT, formed stool twice a day was detected.

– ***D+106 (D+60 post-FMT)*** – molecular genetic study of bone marrow aspirate has confirmed a complete donor chimerism (> 97 %). However, minimal residual disease (+2.3 %) was diagnosed, thus making us cancel immunosuppressive therapy with tacrolimus.

– ***D+114 (D+68 post-FMT)*** – the patient was diagnosed with severe GVHD with skin lesions grade 4, intestinal mucous membranes lesions grade 3. Therefore, tacrolimus administration was resumed at a dose of 1 mg/day.

– ***D+119 (D+73 post-FMT)*** – negative dynamics manifested as increased diarrhea (watery mucous stools up to 17 times/day, at a volume of 500 mL/day). Repeated colonoscopy and fibrogastroduodenoscopy showed pathological changes in the biopsy materials represented by extensive lymphocytic ($CD3^+$) infiltration of the mucous stroma and epithelial lining of the glands (Fig. 1d) with visible foci of lumpy nuclear destruction in apoptotic epithelial cells, subtotal or total decay of some mucous glands (Fig. 1c, e, f). Glucocorticoids (methylprednisolone 2 mg/day and ruxolitinib 15 mg/day) were added to therapy.

– ***D+153 (D+105 post-FMT)*** – positive dynamics manifesting as decreased frequency and volume of stool, decrease of fecal calprotectin levels (from 774 μg/g on D+90 after FMT to 56 μg/g on D+120 after FMT). Repeated fibrogastroduodenoscopy and fibrocolonoscopy were performed. The results of biopsy histology showed pathological changes in the colon mucosa characterized by focal scanty lymphocytic intraepithelial infiltration and focal nuclear destruction of epithelial cells, however without visible gland destruction having been interpreted as mild acute GVHD (Fig. 1b).

– ***D+244 (D+190 post-FMT)*** there are no clinical signs of overlap syndrome at skin, mucous membranes, or intestines; formed stool once a day. The appetite is satisfactory, weight gain – 5.5 kg (the weight loss was 20 % during previous overlap syndrome, with minimum weight of 40 kg).

– D+304 (D+260 post-FMT) – the patient remains in stable clinical and hematological remission. There are no signs of intestinal GVHD. The complete follow-up period for the patient exceeds 5 years.

Results

Dynamics of fecal microbiota during FMT and therapy of intestinal GVHD

After FMT, an increase in the total bacterial mass was observed from 1.0×10^9 to 7.0×10^{11} CFU/g, reached maximum values of 1.0×10^{13} CFU/g at D+70 after FMT (Fig. 2d). Along with the increase in total bacterial number, the species diversity of intestinal microbiota was also enlarged, with Shannon index increased from 2.0 to 3.6 within 3 days after FMT procedure (Fig. 2c).

The patient's microbiota before FMT was mainly represented by bacterial *Proteobacterota* (51.4 %) and *Firmicutes* (47.1 %) (Fig. 2a). In particular, the opportunistic microorganisms belonged to the following genera: *Klebsiella* (43.9 %), *Enterococcus* (16.4 %), *Streptococcus* (23.6 %), *Lactobacillus* (2.5 %), *Enterobacter* (0.6 %). On the contrary, the donor microbiota was enriched by anaerobic phyla, e.g., *Bacteroidota* (27.7 %), *Bacillota* (60.9 %), *Aktinomycetota* (1.2 %).

From D+3 to D+70 after FMT, the patient's FM was gradually dominated by *Firmicutes* (11.7–55.2 %), *Bacteroides* (10.4–36.3 %), *Actinomycetota* (0.3–2.8 %), with increase in *Faecalibacterium* (up to 29.4 %), *Blautia* (up to 7.8 %), *Roseburia* (up to 9.5 %) (Fig. 3a– d). The presence of *Enterobacter, Enterococcus,*

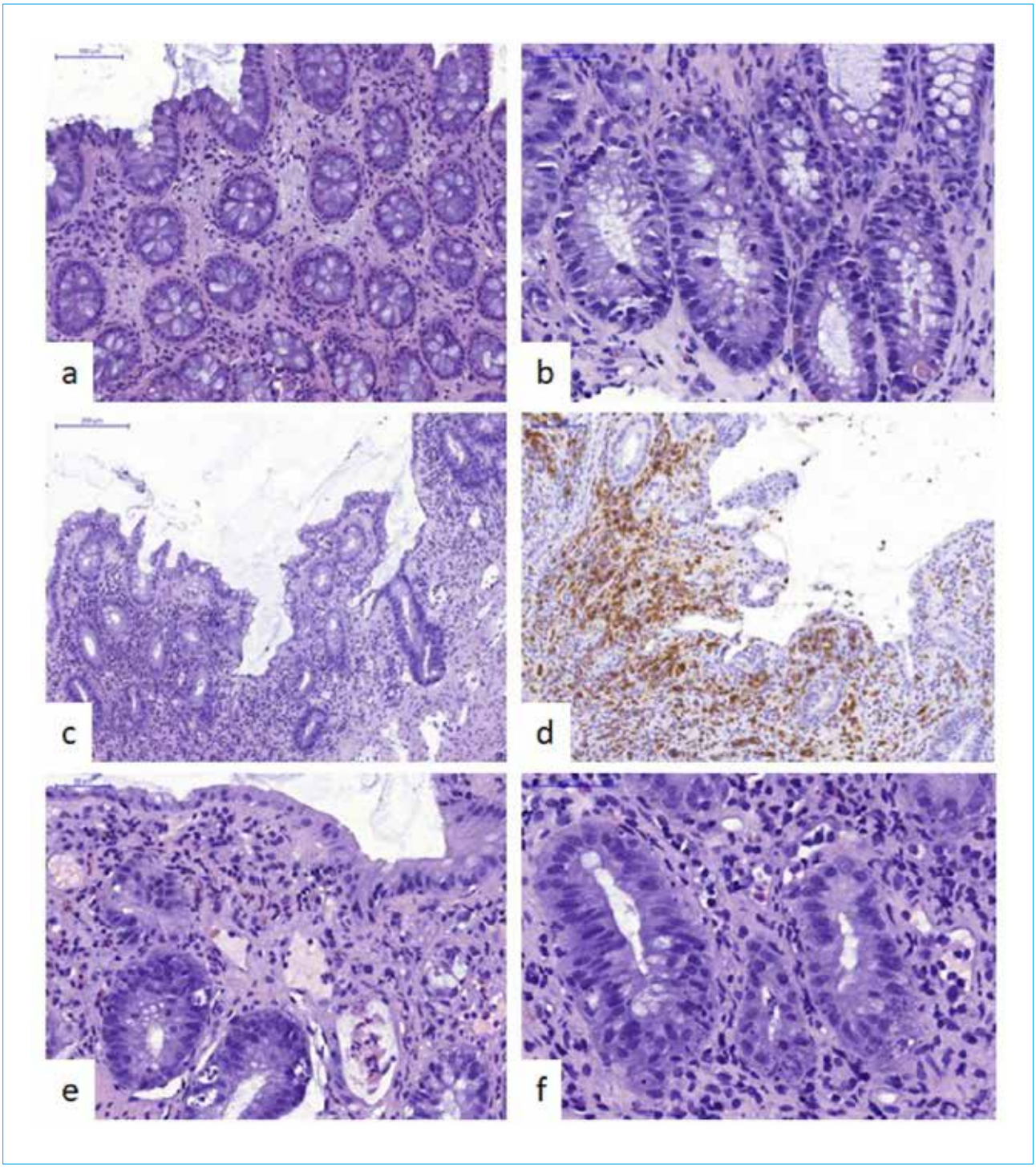

Figure 1. Morphological changes of colonic mucosa membranes at different stages of therapy: a — non-specific reactive changes of mucous membrane on D+25; b — mild GVHD pattern without destruction of mucous glands on D+153; c–f — severe GVHD pattern with massive intraepithelial lymphocytic infiltration and reduction/destruction of glands in the lamina propria of the mucous membrane on D+119; hematoxylin and eosin staining — a, b, c, e, f; immunohistochemical examination for CD3 — d; scale bars are shown in the pictures

Рисунок 1. Морфологические изменения слизистой оболочки толстой кишки на всех этапах терапии: a — неспецифические реактивные изменения слизистой оболочки на Д+25; b — слабовыраженная РТПХ без деструкции желез слизистой оболочки на Д+153; c–f — тяжелая РТПХ с выраженной внутриэпителиальной лимфоцитарной инфильтрацией и снижением/деструкцией желез в собственной пластинке слизистой оболочки на Д+119; окраска гематоксилином и эозином — a, b, c, e, f, иммуногистохимическое исследование (CD3) — d; увеличение отражено на изображениях в виде мерной шкалы

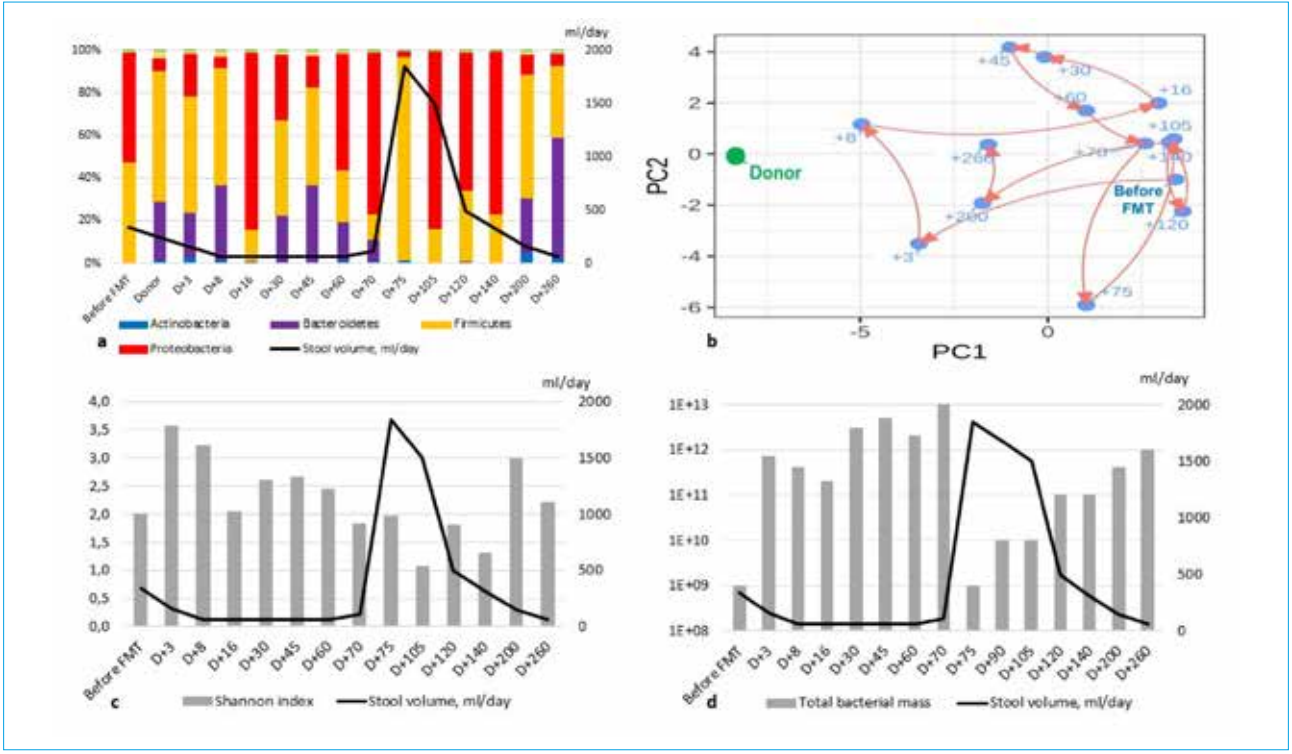

Figure 2. a — relative content of individual bacterial phyla (abscissa — time after FMT, days); b — recipient microbiota profile; c — Shannon species diversity index; d — dynamics of total bacterial mass (log10 CFU/g); for c and d: abscissa — time after FMT, days; ordinate — microbiota parameters (left axis), stool volume (right axis)

Рисунок 2. a — относительное содержание отдельных типов бактерий (по оси абсцисс — сроки после ТФМ, дни); b — профиль микробиоты реципиента; c — индекс видового разнообразия Шеннона; d — динамика общей бактериальной массы (log10 КОЕ/г); для c и d: по оси абсцисс — сроки после ТФМ, дни; по оси ординат — параметры микробиоты (левая ось), объем стула (правая ось)

Klebsiella, and *Lactobacillus* genera has been reduced significantly (Fig. 3e–h). Composition of the recipient's microbiota was nearly close to the donor values on days 3–8 after FMT (Fig. 2b).

Early after withdrawal of immunosuppressive therapy, the patient developed acute GVHD of gastrointestinal tract (at D+75 after FMT), followed by a sharp decrease in total bacterial mass to pre-transplant values (1.0×10^9 CFU/g) and depletion of microbiota diversity on species level (Shannon index decreased to 2.0) (Fig. 2c, d). The differences between microbiota composition of donor and recipient were maximal on days +70 to +140 after FMT (Fig. 2b). We observed a repeated increase in *Proteobacterota* (up to 83 % on D+105 after FMT) (Fig. 2a), an increased proportion of *Klebsiella, Enterococcus, Enterobacter, Lactobacillus* (Fig. 3e–h). The level of *Bacteroidota* decreased from 10.5 to 0.3 %, the number of *Bacillota* genera (*Faecalibacterium, Blautia, Roseburia*) decreased (Fig. 3a–d). A similar microbiota profile was maintained until D+140 post-FMT. Upon recovery from acute gastrointestinal GVHD, the Shannon index showed an increase to 3.0 (Fig. 2c) with total bacterial mass increased to 1.0×10^{12} CFU/g (Fig. 2d), and the profile of patient's microbiota again resembled the donor parameters over D+200 to +260 after FMT, with domination of *Bacteroidota* (56.7 %), *Bacillota* (33.5 %), and re-appearance of *Actinomycetota* phylum (2.5 %). Meanwhile, the levels of *Proteobacterota* decreased to 5.6 % (Fig. 2a).

Time-dependent changes of organic acid levels in the course of FMT and intestinal GVHD therapy

Due to sufficiently changes of fecal microbiota, over time after FMT, one could expect altered levels of PUFAs and bile acids in blood plasma. Prior to FMT, the patient exhibited low levels of secondary bile acids (deoxycholic acid (DCA) — 9.2 μg/mL, lithocholic acid (LCA) — 3.3 μg/mL), and tertiary bile acid (ursodeoxycholic acid (UDCA) — 0 μg/mL). After FMT, a significantly increased content of bile acids was observed, with maximum values at D+45 after FMT. From D+70–75 after FMT, the levels of DCA and UDCA were sharply decreased thus corresponding to

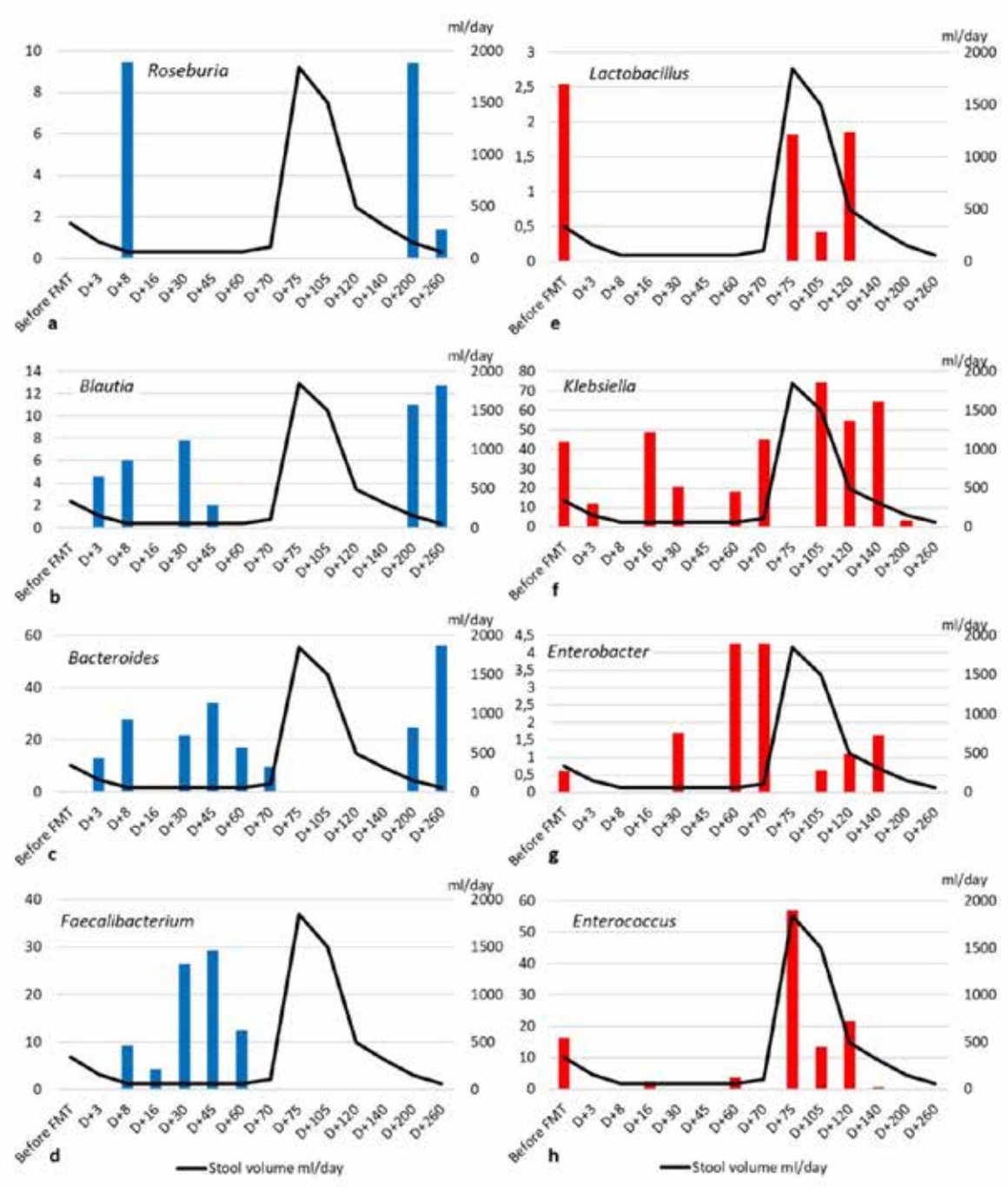

Figure 3. Dynamics of distinct bacterial genera in the course of disease treatment: a — *Roseburia*, b — *Blautia*, c — *Bacteroides*, d — *Faecalibacterium*, e — *Lactobacillus*, f — *Klebsiella*, g — *Enterobacter*, h — *Enterococcus*; abscissa — terms after FMT, ordinate — ratio of distinct genus to the total bacterial mass (left axis); stool volume, mL/day (right axis)

Рисунок 3. Динамика отдельных бактериальных родов в ходе исследования: a — *Roseburi*a, b — *Blautia*, c — *Bacteroides*, d — *Faecalibacterium*, e — *Lactobacillu*s, f — *Klebsiella*, g — *Enterobacter*, h — *Enterococcus*; по оси абсцисс — сроки после ТФМ, по оси ординат — отношение отдельного рода к общей бактериальной массе (левая ось), объем стула, мл/сут. (правая ось)

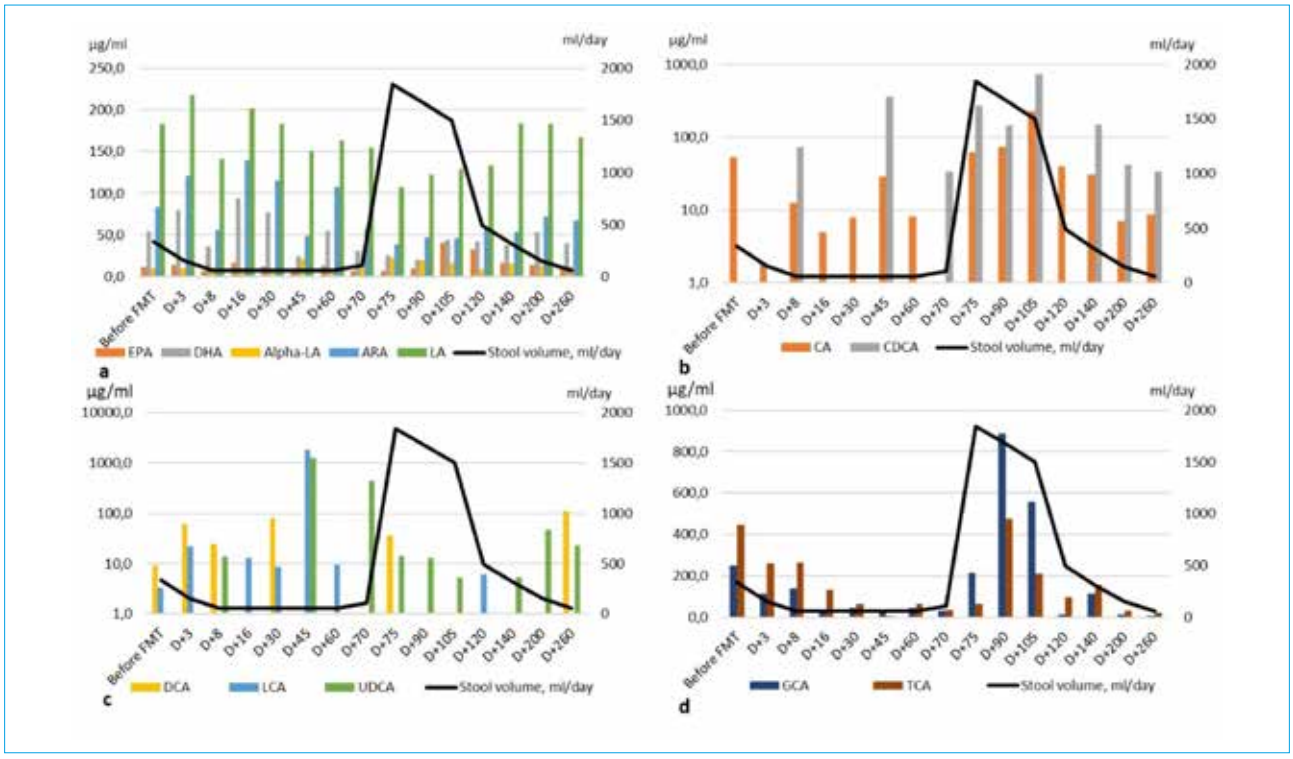

Figure 4. Time-dependent changes of organic acids in blood plasma of the patient: a — polyunsaturated fatty acids, b — primary bile acids, c — secondary bile acids, d — paired bile acids; abscissa — terms after FMT; ordinate — plasma bile acid content, μg/mL (left axis), stool volume, mL/day (right axis)

Рисунок 4. Динамика органических кислот в плазме крови в ходе исследования: a — полиненасыщенные жирные кислоты, b — первичные желчные кислоты, c — вторичные желчные кислоты, d — парные желчные кислоты; по оси абсцисс — сроки после ТФМ, по оси ординат — содержание желчных кислот в плазме, мкг/мл (левая ось), объем стула, мл/сут (правая ось)

the period of acute gastrointestinal GVHD (Fig. 4c). By the end of observation period (D+200–260 post-FMT), upon resolution of acute GVHD, the level of secondary bile acids was restored again.

It should be noted that increased volume of diarrhea was accompanied by an increased level of paired and primary bile acids, such as glycocholic acid (GCA), taurocholic acid (TCA), cholic acid (CA) and chenodeoxycholic acid (CDCA) (Fig. 4b, d).

There is a direct relationship between total bacterial mass and the level of UDCA, and an inverse relationship between total bacterial mass and the level of primary and paired bile acids. An increase in UDCA synthesis was associated with expansion of such bacterial families as *Ruminocaccaceae, Bacteroidaceae*. On the contrary, the reduced levels of *Enterobacteriaceae* and *Enterococcaceae* were revealed.

After FMT, the levels of omega-3 PUFA (eicosapentaenoic acid, EPA) remained relatively stable. However, before the onset of enteropathy (from D+70), a decreased concentration of the latter is observed. The maximum EPA values were recorded at D+105 after FMT, i.e. with documented positive changes (decreased frequency and volume of stool along with lower levels of fecal calprotectin), 40 days before resolution of gastrointestinal GVHD (Fig. 4a). Omega-6 fatty acids, i.e., arachidonic (ARA), linoleic (LA) acids and omega-3 — docosahexaenoic acid (DHA) also correlate with total bacterial mass and clinical pattern of GVHD: their levels decreased with severity of enteropathy. Omega-3 alpha-linolenic acid (alpha-LA) values showed inverse changes.

Time-dependent changes of immune parameters in the course of FMT and intestinal GVHD therapy

The time dynamics of peripheral blood (absolute leukocyte, neutrophils and lymphocytes count) fully reflects the changes of clinical pattern. An increased level of blood immune cells was registered immediately after FMT, and the minimum values were recorded during acute GVHD and escalation of immunosuppressive therapy (Fig. 5a).

The absolute lymphocytes count showed an increase over the first 3 days after FMT due to T-cytotoxic lymphocytes, T-NK cells and natural killer cells. Meanwhile, the T helper and B cell

counts were at stable levels. From D+8, absolute T lymphocytes count decreased slightly, along with the increase in B lymphocytes.

From D+70 to D+120 after FMT (upon tacrolimus withdrawal), a continuous decline in T lymphocytes was found, in parallel with development of acute intestinal GVHD, probably associated with the escalation of immunosuppressive therapy. At the same time, the maximal contents of B cells have been approached, with a trend towards a decrease in B1 lymphocytes and increase in activated memory B cells (D+90 to D+120 FMT). Starting from D+120 FMT, upon gradual recovery from acute gastrointestinal GVHD, we observed a significant increase in T-cytotoxic lymphocytes, T killer and T helper cells which persisted until D+140 FMT, then followed by gradual decrease until the end of observations (Fig. 5b–d, f). The parameters of humoral immunity were also restored following successful treatment of acute gastrointestinal GVHD (Fig. 5e).

Hence, the time-dependent dynamics of T-cytotoxic lymphocytes, NK cells and natural killer cell populations showed some correlations with total bacterial mass, Shannon biodiversity

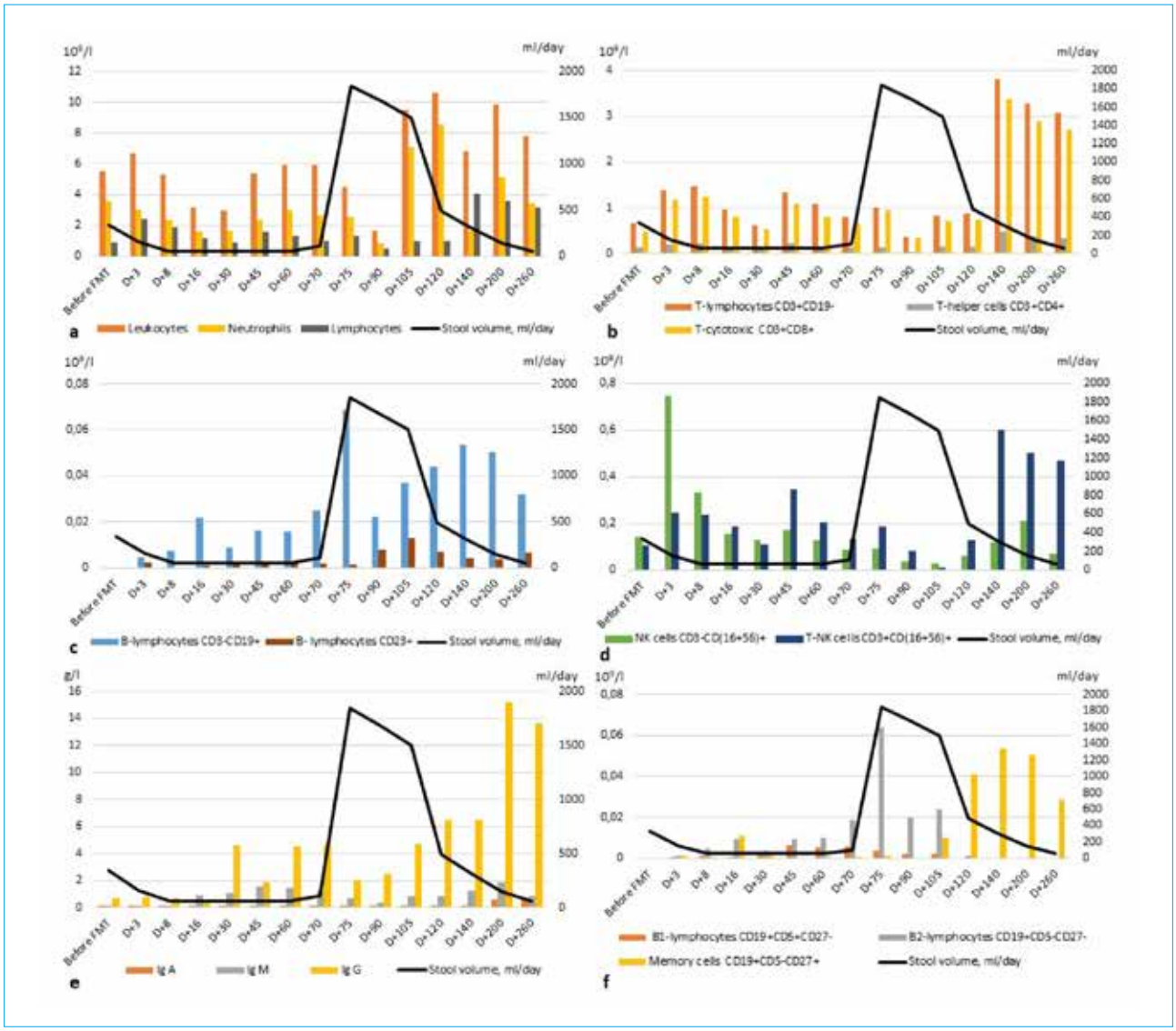

Figure 5. Time-dependent changes of peripheral blood counts, immune cell subpopulations, and humoral immunity: a — peripheral blood cell counts; b — T cell counts, c — B cell counts; d — natural killers; e — serum immunoglobulins; f — naïve lymphocytes and natural killers; abscissa — terms after FMT, ordinate — absolute values (left axis), stool volume, mL/day (right axis)

Рисунок 5. Динамика показателей периферической крови, клеточного и гуморального иммунитета: a — показатели периферической крови, b — Т-лимфоциты, c — В-лимфоциты, d — натуральные киллеры, e — сывороточные иммуноглобулины, f — наивные лимфоциты и натуральные киллеры; по оси абсцисс — сроки после ТФМ, по оси ординат — абсолютные значения (левая ось), объем стула, мл/сут (правая ось)

index of gut microbiota, and the level of secondary bile acids in blood plasma. In addition, a coincidence was found in the dynamics of primary bile acids and B-lymphocytes, the values of which increased during the development of acute intestinal GVHD.

Discussion

Variability of human microbiome may lead to changes in bacterial genetic composition (metagenome) thus causing sufficient changes in metabolic network (metabolome). Over recent years, multiple researchers have applied the so-called metabolomic approach in order to study pathogenesis of different disorders [16, 17]. The key concept of metabolomics presumes that the changes occurring in transcriptome, genome or proteome are reflected in changing concentrations of metabolites in biological fluids and tissues, thus leading to changes in the metabolome [18]. Commensal intestinal microorganisms are producers of short chain fatty acids, which are necessary for maintaining epithelial barrier, regulating innate immune cells, and the antigen-specific adaptive response mediated by T and B lymphocytes [15]. In particular, butyrate suppresses the production of proinflammatory cytokines (IL-6, IL-12, TNF-α, MCP-1) induced by lipopolysaccharides and regulates T cell differentiation [16]. Propionate may inhibit NF-kB signaling, also reducing the levels of proinflammatory cytokines [20]. Over post-transplant period, the patients who have developed GVHD show scarcity of some bacterial species in fecal microbiota, along with predominance of opportunistic flora (*Enterobacteriaceae, Streptococcaceae*) [21, 22]. In the present clinical case, FMT was followed by an increase in total bacterial mass, reduction of *Enterobacteriaceae, Streptococcus, Lactobacillus, Enterococcus*, and re-appearance of *Bacteroides*, propionate-producing bacteria, along with increased relative number of butyrate-producing bacteria (*Faecalibacterium, Blautia, Roseburia*). In parallel with these changes of intestinal microbiota, we have found evident activation of T-cytotoxic lymphocytes and differentiation of naïve B lymphocytes into memory B cells.

In our case, early withdrawal of immunosuppressive therapy led to the development of intestinal GVHD accompanied by loss of bacterial diversity and increase in opportunistic flora. As GVHD resolved, the gut microbiota was enriched again. It should be noted, however, that the microbiota restoration proceeded to the donor composition rather than to the pre-transplant parameters. In parallel to recovery of bacterial mass and diversity of microbiota, we have observed differentiation of T and B lymphocytes into T-cytotoxic lymphocytes, T helper and memory B cells, respectively.

In addition to the short chain fatty acids production, fecal microbiota is also involved in metabolism of bile acids by modifying primary bile acids synthesized in the liver into secondary ones. Biles acids regulate the metabolism of lipids, glucose, and the synthesis of bile acids in the liver via activation of specific receptors, with farnesoid X FXR and TGR-5 protein-associated receptors being the most

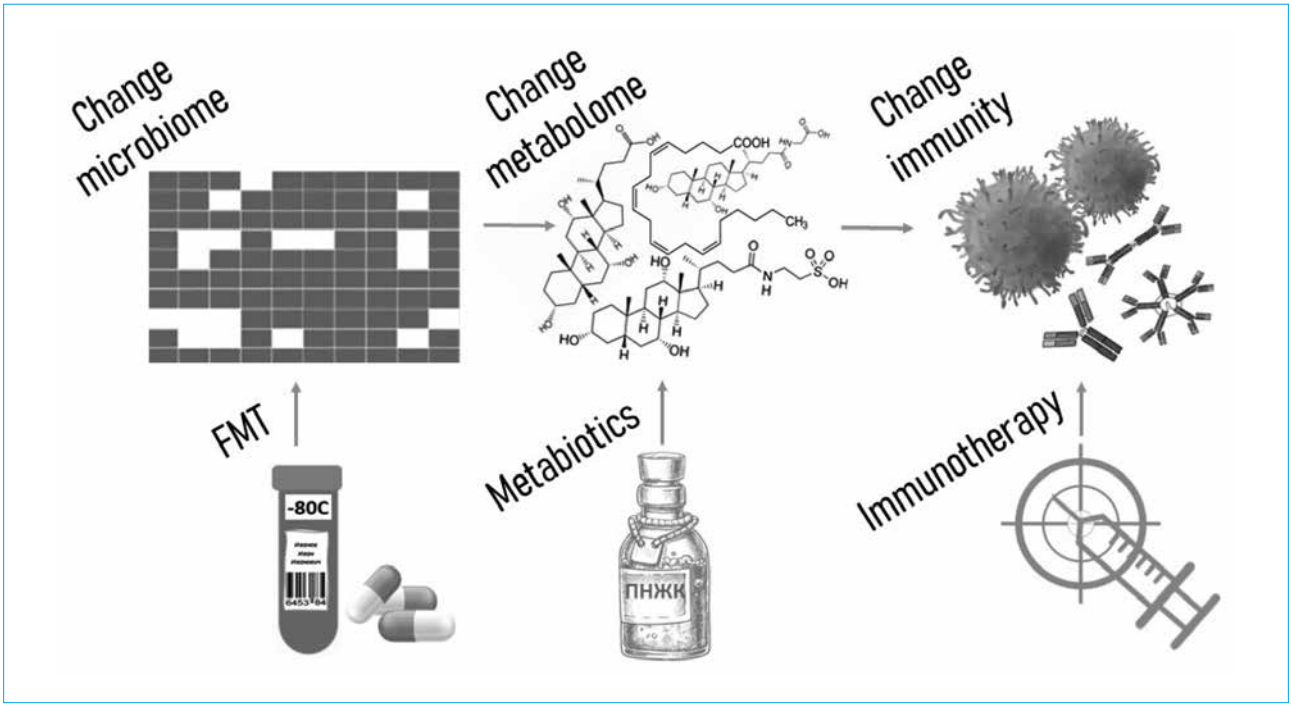

Figure 6. Interrelations between microbiome, metabolome, and changes of immune system

Рисунок 6. Связь между микробиомом, метаболомом и изменениями в иммунной системе

significant [23]. Activation of intestinal FXRs induces transcription of several genes encoding proteins involved in protecting the intestinal mucosa through tight junctions. A decrease in bile acids concentration leads to bacterial overgrowth syndrome and increased bacterial translocation into bloodstream [23, 24].

Our study clearly demonstrates a correlation between bile acids levels and total microbial mass. The levels of primary bile acids (glycocholic and taurocholic) increased with fecal microbiota depletion, thus corresponding to pre-FMT time point and the period of acute GVHD. The inverse pattern was observed for secondary and tertiary bile acids (ursodeoxycholic, deoxycholic and lithocholic). With increased production of secondary bile acids, appropriate T cell dynamics was noted (increase in T-cytotoxic lymphocytes, T helpers).

Basing on modern research concepts, it becomes evident that the microbiome plays an important role in maintaining the main pool of organic acids, and, consequently, homeostatic state of human immune system. Molecular profile of metabolome, as well as immune system parameters, could be directly influenced both by means of medications (metabiotics and immunotherapy) and via changes in the microbiome (Fig. 6).

This theory justifies the development of targeted pre- and probiotic medications [24–26]. However, the practice of long-term microbiome modification has a significant advantage over the short-term effects of metabolic or immune replacement therapy, due to transient effect achieved in the latter case. Moreover, we believe this is the path to personalized medicine for the future. The method of supplementing or removal of certain microorganisms may enable us to impact distinct links of immune response.

Conclusions

The present clinical case demonstrates the efficiency of fecal microbiota transplantation in resolving the issues of antibiotic resistance, decolonization of pathogenic microorganisms, and prevention of systemic infections after hematopoietic stem cell transplantation. Fecal microbiota transplantation, along with immunosuppressive therapy, also helps to resolve the problems of graft-versus-host disease therapy. Impaired composition of intestinal microbiome leads to changes at metabolic level (short chain fatty acids and bile acids) in blood plasma.

The intestinal microbiota, through the products of its metabolism, is able to influence immune responses in the body, promoting the activation and differentiation of lymphocytes, which can serve as part of therapy aimed at early reconstitution of the immune system and the prevention of graft-versus-host disease in the post-transplant period.

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