Gut microbiome in hematopoietic stem cell transplantation: patient- and treatment-related factors

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Summary

Intensive cytostatic therapy is applied as a standard treatment in malignant disorders of hematopoiesis. Conditioning treatment before allogeneic hematopoietic stem cell transplantation (HSCT) causes both acute cellular immune deficiency and severe damage of gut epithelium, and massive antibacterial therapy lead to profound alterations of gut microflora composition. The aim of this review article was to specify environmental, genetic and treatment-related factors causing changes of gut microbiota in the course of intensive cytostatic therapy, delineating possible approaches to normalization of gut microbiome in HSCT. We discuss early experiments with germ-free organisms, describe common views on the "normal" human gut microbiota, its variability, and changes depending on age, dietary background and genetic predisposal between the main classes of gut microbiota, i.e., anaerobic *Clostridia*, and mostly aerobic *Bacteroides, Proteobacteria*. Changed composition and decreased biodiversity of gut microflora is regarded as a regular consequence of cytostatic and antibacterial therapies during the HSCT procedure. Role of enteric leakage, and effects upon immune system of host are considered in view of graft-versus-disease risk, as well as anti-cancer effects associated with altered composition of intestinal microbiota. Some genes affecting gut microbiota are discussed, e.g., effects of ATG16L1, PD-1, FUT2 and some other gene variants which may alter efficiency of HSCT.

Potential role of multiple gut viruses (virome) is known to much lesser degree, due to relative lack of data derived from next-generation sequencing (NGS) of bacteriophages and eukaryotic cell viruses.

In conclusion, many facts concerning gut microbiota require specific evaluation in human patients. E.g., a number of works was performed in order to correct altered gut microbiota in various intestinal syndromes, including specific probiotic strains of *Lactobacteria, Bifidobacteria, Faecalibacterium prausnitzii*, and more recently, fecal microbiota transplantation, also in the post-HSCT dysbiosis. The main issue is that, when dealing with complex bacterial network of microbiota in clinical settings, we still do not know what exact microbial species (or classes) are producing effector molecules which modify immune response causing severe GvHD or changing the antitumor effects of immune therapy. To compare them with healthy subjects, the normal ranges should be established for distinct classes of intestinal microbiota within different age groups.

Keywords

Microbiome, intestinal, gut bacteria, virome, hematopoietic stem cell transplantation, cytostatic therapy, antibacterial treatment, microflora suppression, gut microbiota transplantation.

Introduction

Intensive cytostatic therapy is applied as a standard treatment in malignant disorders of hematopoiesis. E.g., antitumor chemotherapy of leukemias is performed in several steps: remission induction, consolidating and supportive therapy. In cases of high-risk relapse, the patient is subject to allogeneic hematopoietic stem cell transplantation (HSCT), in order to inactivate residual cancer cells. During last 30 years, allogeneic HSCT was used in more than a million of cancer patients. As first step of treatment, they receive high-dose cytostatic therapy (conditioning treatment) which is usually combined with anti-infectious therapy. The conditioning therapy causes both acute cellular immune deficiency which recovers within months and, even, years [1]. Moreover, severe damage of oral and intestinal epithelium develops after intensive cytostatic treatment, and massive antibacterial therapy lead to alterations of gut microflora composition [2]. Pathogenic bacteria and their products migrate to blood plasma and may cause septicemia with detection of microbes and viruses in blood and on mucosal surfaces.

Allogeneic transplantation is generally performed from HLA-compatible familial or unrelated donor and is often accompanied by autoaggressive graft-versus-host disease (GVHD), an inflammatory epithelium lesion which also contributes to immune alterations and intestinal dysfunction. Such triple effect of cytotoxic drugs, allogeneic immune interactions, and immune deficiency should also change intestinal microbiota and its feedback effects upon host organism, including severe autoaggressive reactions [3].

The aim of our review is to specify biological and treatment-related factors causing changes of gut microbiota in the course of intensive cytostatic therapy and to delineate potential approaches to normalization for intestinal microbiome in such patients.

Previous experience with germ-free mice

The story began 50 years ago, when "gnotobiotic" (germ-free) mice showed longer survival after irradiation at gut-damaging doses, compared to animals with normal gut microflora [4]. In a later study, the dogs subjected to lethal irradiation and bone marrow transplantation treated with antibacterial drugs proved to alleviate posttransplant complications after gut decontamination [5]. In 1980's, total or selective gut decontamination (e.g., neomycin, polymyxin B amphotericin B) to prevent posttransplant infections (and maintain colonization resistance) was implemented into routine practice of hematopoietic stem cell transplantation [6]. Modern schedules for gut decontamination in cytopenic and immunocompromised patients include different antibacterial, antifungal and antiviral drugs [7]. Prophylactic monotherapy with levofloxacin also seems to decrease rates of infection after intensive chemotherapy in cancer [8]. Fluoroquinolone- containing schedules and other conventional gut decontamination protocols seem to be suboptimal under current epidemiological environment [9]. These approaches should be directed for sparing necessary microbial diversity in gut microbiota and minimize risk for antibiotic-resistant infections.

"Normal" and changed human gut microbiota

Intestinal microbiota consists of myriads microorganisms, comprising a dynamic biological system functioning within a host organism. Human gut microbiota includes >1000 known microbial and fungal species [10]. Gut virome also contains hundreds viral species, both, phages and vertebrate viruses [11]. Over last years, possible role of the microbiota variety is described in a series of extensive review articles [12]. Generally, the major massive of intestinal microbiota may be classified into several big classes: anaerobic *Clostridia* and mostly aerobic *Bacteroides, Proteobacteria*. Anaerobic clostridial species seem to perform a big deal of metabolic events in normal state, producing some essential metabolites for the host organism, e.g. short-chain fatty acids necessary for enterocyte survival and regulating immune effects in the host. Common changes in main classes of microbiota are associated with different gastrointestinal disorders, e.g., inflammatory bowel diseases [13].

Enteric permeability, microbial translocation and effector molecules

Viable intestinal microbiota produces big amounts of biologically active compounds which may under certain conditions penetrate from intestinal lumen via enteric wall to blood and lymph vessels. Live intestinal bacteria are thought to cross enteric/blood barrier in cases of intestinal damage caused, e.g., by intensive cytostatic treatment, or in severe immune deficiency as it is seen in AIDS patients being a sign of the so-called bacterial translocation [14]. Bacterial components and metabolites in blood serum are known clinical markers of the patients with septicemia [15]. E.g., lipid A (lipopolysaccharide, LPS) is a component of endotoxin from Gram-negative bacteria, being detectable in blood plasma or urine. The LPS presence in serum suggests blood contamination with these bacteria or their fragments. Other plasma markers of septicemia are produced by the host cells (soluble CD14; LPS-binding protein etc.). LPS in clinical material is detected by *in vitro* test with *Limulus* lysate, whereas lipid A may be found and quantified by means of ELISA or mass-spectrometry.

The 2,3-indoxyl sulfate (IS) is a useful marker of a gut dysbiosis, being produced by gut bacteria from tryptophan [16]. It is determined quantitively in blood plasma or urine by means of high-performance liquid chromatography/mass spectrometry. High IS amounts in blood are found in severe infections, intestinal dysbiosis, leakage of intestinal or hepatic barriers. Increase in other microbial metabolites, p-cresyl sulfate and trimethylamine-N-oxide is of similar diagnostic significance. By the contrary, decreased blood citrulline may be a sign of intestinal damage, since it is synthesized by enterocytes [17].

Microbiota and immune response: APC and Th17 T cell network

Microbial and viral antigens, while penetrating mucosae, regional microvessels and lymph nodes, elicit local and regional polyclonal B- and T cell-mediated immune responses, thus being a key factor of normal maturation and functioning of immune system. Important role of intestinal microflo-

ra in development and tuning of general immune response in humans, especially, in childhood, is extensively discussed in a number of review articles [18].

The mechanisms of immune maturation proceed via Th17 and a chain of other signaling factors. Composition of gut microbiota and HLA antigens in host organism are also mutually dependent, both in normal and diseased persons, at least, in children, upon education of their immune system [19].

Hence, there are bidirectional relations between intestinal microbiota and the host immune system which may sufficiently modify the gut microbiome, along with immediate effects of cytostatic therapy, as well as long-term clinical outcomes, especially during hematopoietic stem cell transplantation (HSCT), due to minor differences in HLA antigens between donor and recipient.

Individual diversity of gut microbiota with age and dietary factors

During pregnancy, fetal intestine is sterile, being influenced by a number of external and internal factors [20, 21]. The extrinsic factors are – geographic area, maternal and surrounding environment bacteria, the way of childbirth – natural or by caesarean section, hygiene measures, feeding habits, drug therapies. As usual, colonizing bacteria derive from the mother – mainly vaginal and intestinal microbiota, breast milk and surrounding environment [22].

The intrinsic factors include neonatal genetics, bacterial mucosal receptors and interactions, intestinal pH and secretions, and immune response [24, 25].

One of the most important factors during the first months of life affecting the qualitative and quantitative composition of the microbiota is the fact of breast-feeding, which in addition to the energy function, provides immunoregulation [26], the digestive system functioning due to the presence of growth factors, cytokines, immunoglobulins and digestive enzymes in its composition [27].

During the first and second year of life, differences between breast- and formula-fed infants are lost [28]. But short- and long-term effects of breast-feeding are much better in comparison to formula-fed infants reducing the incidence of allergic and autoimmune diseases [29], inflammatory bowel diseases, cardiovascular diseases, obesity, type-2 diabetes [30, 31].

In the future, microbiota composition changes and its functional activity depend on nutrition features [32]. For example, the commitment to a Western diet, which typically consist of red meat, animal fat, high sugar and low fiber food, leads to an increased number of *Bacteroides* phyla (mainly mucin-degradating bacteria) and *Ruminococcus* [33], reduced number of obligate bacteria, especially in the elderly [34]. While diet rich in fiber correlate with larger bacterial diversity [35] and provide more functional microbiota activity, the degree of immunoregulation and cancer prevention [36].

One of the important questions is the impact of probiotics in the setting of availability to change microbiota characteristics and functioning is still controversial and depends on diagnosis and initial bacteria profile. Probiotics are defined as «live microbial food supplements or components of bacteria which have been shown to have beneficial effects on human health» and generally contain bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium*. It is shown that probiotics may have positive influence on immune functions, blood cholesterol decrease, vitamin synthesis, anti-cancerogenesis and anti-bacterial effect [37]. On the other hand,
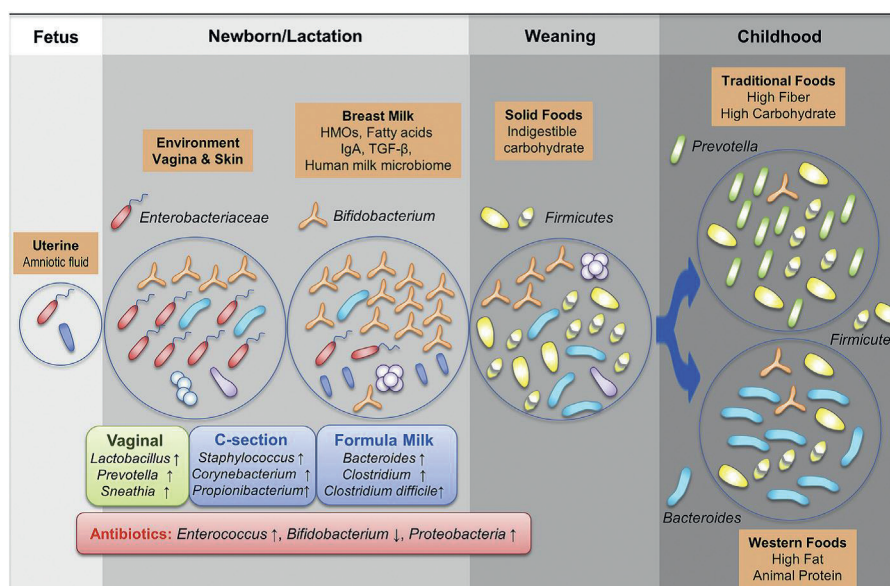

Figure 1. Overview of age-dependent human gut microbiota colonization (Note enrichment with anaerobic *Firmicutes* with high-fat and protein diet). Diversity of the gut microbiota increases with age until it becomes a stable adult microbiota. (Tanaka, Nakayama, 2017)

there is data indicating that probiotics are unable to colonize intestine for a long period of time, and have less efficacy in treatment of antibiotic associated diarrhea [38].

Some specific host-microbiota relations were found by a joint team from Netherlands and Russia who performed metagenomic sequencing in 1,514 subjects [39]. Using GWAS approach, they have found significant associations of 9 human genome loci with microbial taxonomies and 33 loci with microbial pathways, including genome-wide significance for the C-type lectin molecules CLEC4F-CD207 at 2p13.3 and CLEC4A-FAM90A1 at 12p13, and association of a functional LCT SNP with the *Bifidobacterium* genus ($P=3.45\times10^{-8}$). These findings suggest an evidence of a gene-diet interaction for the regulation of gut *Bifidobacterium* population.

However, an extensive genotype and microbiome study from the same workers based on the samples from 1,046 healthy individuals of different ancestry who shared a relatively common environment has shown that the host genetics does not sufficiently contribute to the microbiome composition [40]. Important similarities are found in the microbiota composition from genetically unrelated individuals who have common household. An estimated value of >20% of the inter-person microbiome variability is shown to be associated with dietary factors, drugs and individual anthropometry.

Indeed, despite sufficient effects of some gene variants, an impact of non-heritable factors, such as diet, seems to predominate the effects of host genetic background [41].

Microbiota affection by antimicrobial and anticancer therapy

Intensive cytostatic therapy is universally accompanied by leucopenia an temporary cellular immune deficiency, thus causing activation of many opportunistic infections [42]. E.g., the *Clostridium difficile* infection is considered an important factor of intestinal disorders and general immune suppression in childhood [43]. The antibiotic-resistant bacterial strains occur at higher rate in gut, as seen from results of routine bacteriological studies.

Over last years, numerous studies show depletion of distinct bacterial groups as shown by next-generation sequencing. Load and composition of fecal microbiota were studied immediately after treatment in 21 patients, who received broad-spectrum antibiotics such as fluoroquinolones and b-lactams [44]. Fecal samples were collected from all participants before treatment and one week after for microbial load and community composition analyses by quantitative PCR and pyrosequencing of the 16S rRNA gene. The study has shown a decrease in total bacterial load, and ratio of sufficient anaerobic bacteria. At the phylum level, the treatment with antibiotics increased the Bacteroidetes/Firmicutes ratio, as well as at the genera levels, mostly, due to *Lachnospiraceae* and *Blautia* exhaustion.

By contrary, experimental treatment of mice with cyclophosphamide is associated with depletion of *Bacteroidetes* in gut microbiota, along with accumulation of potentially harmful bacteria [45].
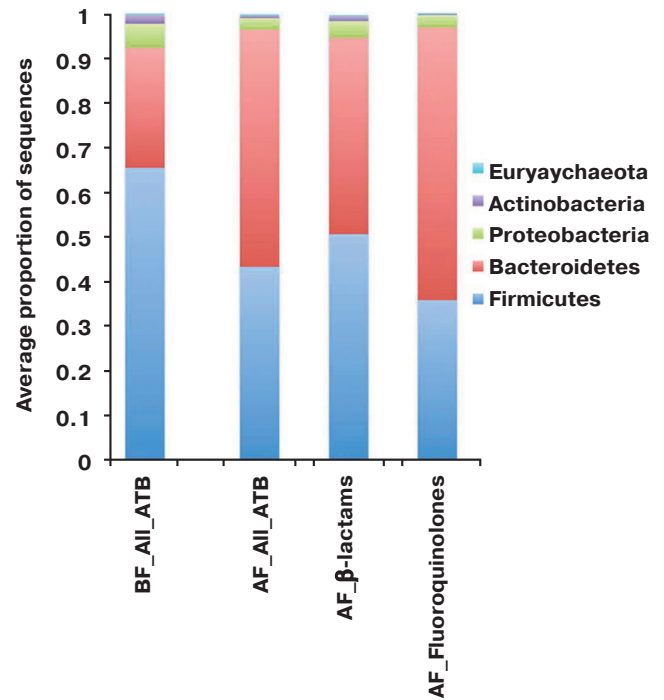

Figure 2. Microbial composition at the phylum level based on 16S rRNA gene sequences. BF = Before treatment; AF = After treatment; ATB = Antibiotics. For all antibiotics N= 21; for b-lactams N = 11; for fluoroquinolones N= 10 (Panda et al., 2017)

However, the question remains open, how combined antibacterial/anticancer therapy (e.g., cyclophosphamide) will affect intestinal microbiota in patients with malignancies and affect clinical outcomes. To this purpose, future clinical studies are required. Disturbed composition of gut microflora may be accompanied by remarkable functional changes of both anti-infectious and antitumor immunity [46].

Gut microbiota and acute GvHD

Acute GvHD is a common and often life-threatening HSCT complication caused by cytotoxic effects of donor T cells against skin and gut epithelium of the patients. GvHD severity may vary from grade 1 (mild reaction) to III-IV. A lot of genetic factors are shown to predispose for severe GvHD. Moreover, some infectious factors seem to cause or modify clinical course of GvHD, such as reactivation of CMV or EBV [1, 42].

Possible role of gut bacteria as a risk factor for GvHD is now questioned. E.g., antibacterial and cytostatic therapy before HSCT causes depletion of *Clostridiales* order, especially, *Blautia* genus. Reduction in these clostridiococci proved to be associated with higher GvHD mortality in these patients [47]. Different changes of gut microbiota and relevant immune mechanisms promoting GvHD in allo-HSCT patients were recently summarized [48, 49].

Host pleiotropic genes: their presumable effects upon gut microbiota

After initial optimism on predisposing role of functional gene variants in various disorders, some cautions appeared, when interpreting possible changes in genetic immune regulation, e.g., after hematopoietic stem cell transplantation. The problem is that, besides numerous gene polymorphisms of protein-encoding segments, one should consider effects of other single-nucleotide polymorphisms (SNPs) in regulatory elements and small molecules, like as microRNAs (miRNAs), and their interactions. Hence, phenotypic effects for most SNPs may be rather blurried and not reproducible when studied in different populations and clinical series, as reviewed by Gam et al. [50].

About 100 genes or allelic variants, mostly those controlling immune functions of Th1, Th2, and Th17 effector populations, were shown to be associated with susceptibility with inflammatory bowel diseases (IBD), as reviewed by Basso et al. [51].

However, at the present time, current GWAS studies allowed to find correlations between genotype and phenotype for a number of pleiotropic human genes which modify quite different disorders, e.g., lipid disturbances and immune diseases [52]. A detailed genotype-phenotype analysis has revealed shared effects common for gut immune disorders (e.g., Crohn's disease, ulcerative colitis, celiac disease etc.), and lipid biology. These genes concern several shared pathways including glycosphingolipid synthesis (e.g. FUT2) and intestinal host-microbe interactions (e.g. ATG16L1).

ATG16L1 and gut pathology

The ATG16L1 gene encodes an autophagy protein which is produced in many cell types, including antigen-presenting cells. Among different polymorphisms, a single variant of ATG16L1 (rs2241880, T300A) may predispose for development of Crohn's disease. The protective ATG16L1 allele encodes threonine at amino acid position 300 (ATG16L1*300T), whereas ATG16L1*300A encoding alanine confers higher risk for development of Crohn's disease [53]. In human intestinal epithelium, the Crohn's disease-associated ATG16L1 coding variant shows impaired capture of internalized Salmonella within autophagosomes. Thus, we propose that the association of ATG16L1*300A with increased risk of Crohn's disease is due to impaired interactions with bacterial and decreased bacterial capture by autophagy.

ATG16L1 in the intestinal epithelium was shown to prevent loss of Paneth cells and exaggerated cell death in animal models of experimental inflammatory bowel disease, and, interestingly, allogeneic hematopoietic stem cell transplantation. The mutant Atg16L1HM mice are more affected by graft-versus-host disease (GVHD) after allo-HSCT. Hence, ATG16L1 seems to keep the intestinal barrier by inhibiting epithelial cell death [54].

An elegant study was performed by Sadabad et al. [55]. The inflamed and non-inflamed sites of ileal mucosa from ATG16L1-typed patients with Crohn's disease were studied, with respect to microbiota composition at these sites. Inflamed ileal tissue of patients homozygous for the ATG16L1 protective allele showed decreased numbers of *Bacteroidaceae* and *Enterobacteriaceae* and increased *Lachnospiraceae*. Upon *in vitro* assays, the monocytes homozygous for the ATG16L1 risk allele showed impaired killing of pathogenic *E.coli* under inflammatory conditions. However, the ATG16L1 allele did not affect the bacterial composition in the non-inflamed ileal tissue. The authors suggest that the host cellular immunity seems to regulate the gut microbiota composition by genetic mechanisms.

Another study [56] has shown that the common GG variant of ATG16L1 interfered with the production of IL-1β, which was highly induced in PBMCs from patients with GG genotype by exposure to pathogenic *E.coli*. The authors have also observed that the T300A variant in patients with CD strongly increases the risk for complicated fistulizing disease, and significantly affects antibacterial responses in vitro. Meanwhile, any studies on the role of ATG16L1 in HSCT are absent in available literature

PD-1 gene

The PD-1 and its ligands (PD-1L) represent a system of costimulatory signal proteins that regulate activation and deactivation of T cells, modulates immune response to infectious pathogens and tissue antigens, thus mediating some autoimmune conditions [57]. PD-1 is encoded by the PDCD1 gene, being expressed on many cell types in humans. Hence, its expression may sufficiently influence both antiinfectious and antitumor response in HSCT patients.

PD-1 is a coinhibitory receptor that is inducibly expressed on T cells and B cells, natural killer T cells, and monocytes. Carriers of the A allele express lower levels of PD-1 receptor on the Treg cells (CD4+CD25+ cells) [58]. The variable G/A site is located in an intronic enhancer (intron 4, position 7,146) within a DNA-binding site for the RUNX1 transcription factor. Appropriate gene variant was called PD-1.3 (rs11568821). The common variant allele A is suggested to contribute to an aberrant transcriptional regulation of PD-1 in SLE and other autoimmune diseases.

However, typing of the PDCD1 gene may be also informative in transplantation settings. I.e., Hoffmann et al. [59] have genotyped the PD-1 variants in 469 seropositive kidney graft recipients and showed a significant correlation between CMV reactivation and PD-1.3 allele A which proved to be associated with CMV infection posttransplant. Interestingly, inclusion of functional IL12B 3'UTR variants increased this association. In other study, the PD-1.3 variant was typed in 1119 kidney recipients and 181 lung recipients [60]. In 481 kidney transplants, the A allele carriers showed less common kidney graft failure than the G homozygotes. Moreover, evaluation of 85 lung recipients has shown similar results, i.e., the A-carriers had longer survival, and better function of transplanted organ. In addition, the, AA recipients had a stronger anti-CMVpp65 T-cell response than the GG-typed patients.

Effects of donor PD-1 variants upon clinical course of post-HSVCT period were presented by Santos et al. [61]. The workers have found an increased risk of grades II to IV graft-

versus-host disease (GvHD) when the grafts were used from the donors homozygous for the A allele of the rs11568821 SNP. Those subjects comprised only 30 cases out of 1500 (ca.1.5%). Meanwhile, the PD-1.3 G>A genotype of the donor was not associated with overall survival or relapse incidence. Hence, the PD-1 gene polymorphism effects seemed to affect, mainly the GVHD immune aspect in this extensive study.

Association between PD-1 variants and sepsis outcomes was also found by Mansur et al. [62] who studied the rs11568821 SNP in 219 patients with severe sepsis. The 3-month mortality proved to be much higher for the GG group than for A allele carriers, with increased scores of multiple organ failure.

A number of novel inhibitors of PD-1 or PD-1 ligand are now introduced into clinical practice. In this respect, certain probiotic gut bacteria are considered a sufficient modifying factor when treating malignancies with these immune checkpoint inhibitory drugs [63].

FUT2 gene

This gene encodes fucosyl transferase, an enzyme adding a fucose residue, thus producing secretor state of H blood group antigen. This surface molecule also serves as a receptor for some intestinal viruses, thus the secretor state of FUT2 largely determines susceptibility to rotavirus and some other gut viral infections [64].

Additional evidence for the FUT2 gene polymorphism as a factor of rotavirus infection was found in the study by Günaydın et al. [65]. Rotavirus-specific antibody titers proved to be significantly higher in persons with secretor FUT2 variants than in non-secretors.

A special meta-analysis (about 10,000 cases) as revealed a strong association between the rs601338 (W154X) in the FUT2 gene [66]. The children with the A allele, which results in a truncated FUT2 protein, had lower risk of diarrhea, presumably, due to decreased numbers of cell receptors for pathogenic viruses (e.g., rotavirus).

To characterize metabolic effects of FUT2 gene polymorphism upon the mucosal ecosystem, a simultaneous assessment of microbiome, meta-proteome and meta-metabolome was performed in 75 endoscopic lavage samples from the cecum and sigmoid from 39 healthy subjects with different FUT2 gene status (rs601338 G>A). The general metagenomic analysis revealed perturbations of energy metabolism in the microbiome from the non-secretor persons, i.e., enhanced carbohydrate and lipid metabolism, altered glycan biosynthesis and depleted amino-acid metabolism. Similar changes were reproduced in mice carrying the *FUT2*(-) genotype [67].

However, the associations between FUT2 secretor genotype and gut microbiota diversity were not confirmed by a recent study performed in 1190 healthy persons since no correlations were revealed for alpha-diversity, or microbial composition assessed by NGS approach [68].

Other human genes potentially changing gut microbiota

Several years ago, the group by Holler et al. [69] has discovered a distinct correlation between certain polymorphisms of TLR and NOD2/CARD gene and high incidence of acute intestinal GVHD after HSCT. These genes encode specific pattern-recognition receptors for bacterial antigens and mediate acute inflammation switched by innate mechanisms. Special studies of intestinal biopsies from the GVHD patients have shown loss of protective CD4 T cells which was more pronounced in carriers of NOD2/CARD15 gene variants [70].

Relations of NOD/CARD system and other gut disorders is also confirmed by the results of Hrnčířová et al. [71] concerning distinct associations between Crohn's disease and some gene variants of CARD15/NOD2 gene.

Associations between NOD/CARD polymorphisms and posttransplant infections were studied by Grube et al. [72]. The authors found a significant association between the presence of donor NOD2 SNP13 (3016_3017insC) and the incidence of septic shock (P <002). In multivariate analysis, donor NOD2 SNP13 appeared as an independent risk factor for the incidence of septic shock after allo-SCT.

In a Chinese study of sepsis cohort, the authors did not found any significant associations for either TLR gene polymorphisms (rs4986790 or rs4986791) with sepsis susceptibility in total analysis in any genetic models [73].

Less significant (minor) differences for other gene variants may be obtained in large study groups, found due to big statistics. E.g., an international group has tested 10,523 IBD cases and 5,726 non-IBD controls by means of GWAS approach using the Illumina technique [74]. The workers have revealed a highly significant association between between Crohn's disease and a missense variant in the zinc transporter solute carrier family 39, member 8 protein (*SLC39A8* Ala391Thr, rs13107325). The association of this SNP with microbiota was assessed in 338 colonic mucosal lavage samples using 16S rRNA sequencing. The Crohn's disease risk allele proved to be associated with altered colonic mucosal microbiome in healthy controls and the patients (p=0.0009). Among major bacterial taxa of colon microbiota, the SLC39A8 Thr391 allele carriers exhibited significant depletion of, e.g., *Coprococcus, Roseburia, Lachnobacteria, Faecalibacterium prausnitzii* and *Ruminococcus gnavus* in Crohn's disease patients.

Pro- and anti-cancer effects associated with altered composition of intestinal microbiota

Over last years, abnormal intestinal microbiota is recognized as a factor of cancer treatment efficiency and, especially, in cancer immunotherapy [75]. Potential associations between altered gut microbiota and clinical outcomes of malignancies are studied.

E.g., colonic presence of *Fusobacterium nucleatum* correlates with increased risk for colorectal cancer, and its overpresentation in stool is shown to be associated with higher resistance of this tumor to chemotherapy [76].

In murine experiments, it was shown that prevalence of some intestinal Gram-positive bacteria may increase efficiency of cytostatic tumor treatment, and, *vice versa*, antibacterial therapy eliminating Gram(+) microbes caused a decreased response of the tumors to cyclophosphamide treatment [77]. The authors suggest this biological effect to be mediated by the Th17 T cell network which is switched by gut microflora-derived antigens. Appropriate clinical study was performed in patients with chronic lymphocytic leukemia (CLL) receiving cytostatic therapy [78]. A subgroup of patients treated with antibiotics against Gram-positive bacteria showed earlier progression of malignancy and decreased long-term survival.

Vice versa, predomination of a bacterial population represented mostly of *Eubacterium limosum* correlated with decreased risk of relapse/progression posttransplant [79]. *Eubacterium limosum* belongs to anaerobic bacteria producing butyric and other short-chain fatty acids which are considered to support viability and functioning of gut epithelium and local immune response [80].

Moreover, many attempts were made to correct or replace the damaged gut microbiota with specific strains of *Lactobacteria, Bifidobacteria, Faecalibacterium prausnitzii*, or other probiotics [81], causing partial clinical responses in the patients with immune disorders. Probable potentiation of immune checkpoint inhibitory (anti-PD-1) therapy will be achieved, using distinct probiotic gut bacteria [63].

Probiotics and fecal transplantation post-HSCT

Fecal transplantation as a tool for microbiota substitution was proposed centuries ago. However, clinical indications for FMT and microbial markers of FMT efficiency were developed within last 2-5 years. These indications are based on the integral assessment of gut microbial spectrum performed by different NGS techniques allowing to and assess ratio between distinct bacterial classes and genera [82].

Fecal microbiota transplantation (FMT) is now proposed as a substitution correcting therapy in the disorders characterized by shifts in microbial species, or other disturbances of the gut microbiota (Crohn's disease, nonspecific ulcerous colitis, persistent *C.difficile* infections). Over recent years, sporadic small studies of FMT after hematopoietic SCT were performed in several patients with steroid-resistant intestinal GVHD by the groups from Japan and Netherlands, showing safety and satisfactory clinical efficiency of the treatment method [83, 84]. At our BMT clinics, we have performed small studies with 11 patients who underwent allogeneic HSCT and suffered with multiresistant microbial infections [85]. In most of the patients treated by FMT, fast clinical response was observed, along with positive dynamics of microbial flora in their stool samples. Our further studies are aiming to extend indications for FMT usage after allo-HSCT,

in order to treat bacterial complications and immune disturbances (i.e., graft-versus-host disease) which sufficiently depends on gut microbiota changes [86].

Appropriate clinical trials on the fecal microbiota transplants (FMT) have been carried out since 2014, according to the *Clinical trials.gov* registry, mostly, in Crohn's disease, non-specific ulcerous colitis, resistant *C.difficile* infection. A total of 46 FMT trials for various clinical indications are registered in this field, mostly for the phase I (safety and tolerance).

Gut virome in normal state and after HSCT

Bacteriophages

Only limited number of works concerns gut virome as a big variety of bacteriophages and eukaryotic viruses living in human cells. A competent review by Columpsi et al. [87] highlighted the issue of the intestinal bacteria and phage equilibrium, probability of eubiosis shifts, due to the phage lytic effects, thus causing health disorders. Moreover, the products of bacterial lysis and viral antigens could potentially trigger some adverse inflammatory modulations. There is a large heterogeneity of phages, which are infecting, mostly, specific bacterial classes and are difficult for appropriate taxonomic classification. Ongoing epidemiological studies of intestinal phages are mostly performed by the NGS method, i.e., parallel sequencing of multiple small fragments of DNA followed by *in silico* alignment and reconstruction of multiple genomes, in order to identify known and novel phage sequences.

A recently described intestinal crassPhage with *in silico* estimated properties, however, with unknown incidence and epidemiological features was described several years by means of modern NGS technique and *in silico* digital characterization of this, previously unknown phage which is probably living in Bacteroides and is detectable in ca. 73-77% of humans , is able to vertical transmission [88]. Moreover, the authors have shown its transmission to the recipient during fecal microbiota transplantation in *C.difficile* infection, thus showing an opportunity of tracing its migration pathways.

Eukaryotic cell viruses

Generally, a number of RNA and DNA viruses living in eukaryotic intestinal cells are detected in normal human gut, including rotavirus, astrovirus, calicivirus, norovirus, hepatitis E, adenoviruses etc. [87]. Moreover, quite recently, multiple "novel" RNA and DNA viruses were identified in gut microbiota by their specific gene sequences, e.g., *Picornaviridae, Coronaviridae, Astroviridae, Parvoviridae* members, using high-coverage NGS approach [89].

Clinical viral infections and reactivation of different intestinal viruses were extensively studied in hematopoietic stem cell and organ transplantation, including adenovirus, bocavirus, coronavirus, human herpesvirus-6, lymphocytic choriomeningitis virus, measles, mumps, metapneumovirus, parainfluenza, rotavirus, etc. [90].

Routine clinical protocols for hematopoietic stem cell transplantation include weekly or bi-weekly multiple PCR

screening for herpesviruses (cytomegalovirus, Herpes simplex, Epstein-Barr virus) and, especially, adenovirus for the first 1-2 months posttransplant which correlate with different life-threatening complications [91]. Virus persistence in blood or stool may require further monitoring of viral load and consider the role of pathogen in intestinal disorders (i.e., prolonged diarrhea, intestinal GVHD). A sufficient role of altered gut virome in HSCT was shown by Legoff et al. [48]. The authors have undertaken a global NGS study of gut microbiota in HSCT patients over different time points and have found increased proportion of picobirnavirus (PBV) sequences in stool of the patients who later developed acute enteric GVHD. The increased PBV levels were revealed both before and up to 1 month posttransplant.

Interestingly, filterable (potentially, viral) substances of gut microbiota may be also effective in fecal transplantation. A German group [92] has used stool samples passed through Seitz filters, thus removing all native microbes, leaving presumably viral particles and some bacterial components. These fluid preparations were delivered to intestines of the patients with *C.difficile* infection and have produced good clinical effect, despite absence of intact bacteria in the fecal transplant. Effect of such treatment, if it will be reproduced, may be dependent on bacteriophages and human viruses present in the cell-free fluid used by the workers.

Potential role of bacteriophages in posttransplant conditions is suggested in the review article by Górski et al. [93]. This paper contains a collection of data on positive immune-mediated effects of intestinal bacteriophages upon intestinal epithelial cells, thus, probably, causing mitigation of graft-versus-host disease in humans. Worthy of note, a special study with FMT in *C.difficile* infection has shown that the donor-derived bacteriophages (specifically, Caudovirales) were expanded to larger degree in the patients responding to FMT than in those who did not [94]. *Vice versa*, the FMT recipients who received donor faeces with higher *Caudovirales* abundance were successfully treated with FMT. Appropriate studies in HSCT setting would be of sufficient value in future, thus evaluating role of the phage component on FMT effects.

Future prospects

Despite good current knowledge on sufficient role of intestinal microbiota in HSCT setting, some issues remain unresolved.

First of all, most studies on positive effects of microbiota were performed in experimental models, thus requiring specific evaluation of these facts in human patients. Secondly, when dealing with complex bacterial network of microbiota in clinical settings, we still do not know what exact microbial species (or classes) are producing effector molecules modifying human immune response leading to severe GvHD or associated antitumor effects of immune therapy. To compare them with healthy subjects, the normal ranges should be established for distinct classes of intestinal microbiota within different age groups. Thirdly, the qualitative and quantitative ranges of normal intestinal microbiota should be specified by classical bacteriological and immunological diagnostics accomplished with multiplex DNA diagnostics (multiplex

PCR and next-generation sequencing) of stool samples from healthy persons. As a result, the new molecular targets could be suggested for improved immune therapy of oncological diseases, especially, in childhood. A special issue bears on proven combined effects of the antibacterial/anticancer therapy (e.g., cyclophosphamide) upon intestinal microbiota, with appropriate consequences for early HSCT complications and risk of relapses in human leukemias, lymphomas and some pediatric malignancies.

Due to absence of notable clinical recovery in HSCT from the probiotics treatment, one may, at this step of clinical research, propose a full-microbiota replacement for treatment of severe intestinal dysbioses, i.e., introduction of normal mixed donor microbiota to the gastrointestinal tract, aiming for rapid recovery of normal microbial composition. Such experimental treatment option is a kind of biotherapy which is now effectively used in the patients with persistent *C.difficile* infection, intestinal bowel diseases etc. Hence, fecal microbiota transplantation is feasible in HSCT patients, first of all in antibiotic-resistant colitis, or steroid-insensitive GVHD. Our pilot data suggest safety and certain clinical efficiency of this approach, however, requiring further observations in larger groups.

Conflicts of interest

None of the authors declare any conflicts of interest.

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Микробиом кишечника при трансплантации гемопоэтических стволовых клеток: факторы, зависящие от пациента и терапии

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Резюме

Интенсивная цитостатическая терапия применяется в качестве стандартной терапии злокачественных новообразований гемопоэтической системы. Кондиционирующая терапия перед трансплантацией гемопоэтических стволовых клеток (ТГСК) приводит как к острому клеточному иммунодефициту, так и к тяжелым нарушениям кишечного эпителия, а массивная антибактериальная терапия ведет к глубоким нарушениям состава кишечной микрофлоры. Целью настоящего обзора было уточнение генетических факторов, внешних воздействий и терапевтических факторов, вызывающих изменения кишечной микробиоты в процессе интенсивной цитостатической терапии, обозначение возможных подходов к нормализации кишечного микробиома при ТГСК. Обсуждаются ранние эксперименты с безмикробными животными, описываются общепринятые взгляды на «нормальную» микробиоту кишечника человека, ее вариабельность и изменения, зависящие от возраста, диеты и генетической предрасположенности по основным классам кишечной микробиоты, т. е., анаэробных *Clostridia*, и более аэробных *Bacteroides, Proteobacteria*. Измененный состав и снижение биоразнообразия кишечной микробиоты рассматривается в качестве регулярного следствия цитостатической и антибактериальной терапии в период ТГСК. Роль порозности кишечной стенки и соответствующие эффекты на иммунную систему организма-хозяина рассматриваются в аспекте риска реакции «трансплантат против хозяина», а также возможных антирецидивных эффектов при лейкозах, связанных с изменениями состава кишечной микробиоты. Обсуждаются некоторые гены, влияющие на кишечную микробиоту, например – влияние *ATG16L1, PD-1, FUT2* и других генных вариантов, которые могут влиять на эффективность ТГСК.

Потенциальная роль многочисленных кишечных вирусов («вирома») известна в значительно меньшей степени, в связи с относительной нехваткой данных, полученных путем секвенирования следующего поколения (NGS) бактериофагов и вирусов эукариотических клеток.

В заключение отмечено, что многие факты о кишечной микробиоте требуют особой оценки у человека при его лечении. Проведен ряд работ, направленных на коррекцию измененной кишечной микробиоты при различных кишечных синдромах, в том числе – с использованием отдельных пробиотических штаммов *Lactobacteria, Bifidobacteria, Faecalibacterium prausnitzii*, и в последнее время – трансплантации фекальной микробиоты, в том числе и при дисбиозе после ТГСК. Основная проблема состоит в том, что при анализе сложных взаимодействий бактериальной микробиоты в клинических условиях мы еще не знаем, какие именно микробные виды (или классы) продуцируют эффекторные молекулы, которые модифицируют иммунный ответ, ведущий к тяжелой РТПХ или изменяющий противоопухолевый ответ иммунотерапии. Для соответствующих сравнений со здоровыми людьми следует устанавливать нормальные области значений для конкретных классов кишечной микробиоты в различных возрастных группах.

Ключевые слова

Микробиом, кишечный, кишечные бактерии, виром, трансплантация гемопоэтических стволовых клеток, цитостатическая терапия, антибактериальное лечение, подавление микрофлоры, трансплантация кишечной микробиоты.