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Stem cell transplantation - GvHD - Section 2

The role of the intestinal microbiota in GvHD

Christoph K. Stein-Thoeringer,[1] Marcel R.M. van den Brink[1,2,3]

[1]Department of Immunology, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, USA; [2]Weill Medical College of Cornell University, New York, USA; [3]Adult Bone Marrow Transplant Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Take-home messages

- Profound perturbations of the intestinal microbiota have been discovered in patients undergoing allo-HSCT.
- Antibiotic induced loss of bacterial diversity and shifts in the microbiota are significantly associated with allo-HSCT related morbidity and mortality.
- Microbiota modulation by narrow-spectrum antibiotics, selected microbial ecosystems and metabolites may induce superior benefits regarding survival and gastrointestinal health.

Introduction

The impact of the intestinal microbiota on health and disease has become increasingly clear in the last decade, and imbalances in the gut microflora are highly relevant for a variety of diseases.[1] The human body is colonized by 10^{13} - 10^{14} microbes, and the vast majority resides in the gut with a biomass of mainly anaerobic bacteria.[2] The gut contains also archaea, eukarya and viruses, but their clinical relevance has been less studied. Over 1,200 different bacterial species have been identified in the human intestines, and each individual is host to a set of at least 160 species with a predominance of the bacterial phyla Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria and Verrucomicrobia[3]. Environmental factors like xenobiotics, especially antibiotics, or diet, host genetics and the immune system shape an individual's microbiota.[4,5] In a bidirectional manner, the intestinal microflora, innate and adaptive immunity co-develop after birth and cross-talk during life to achieve homeostatic balance between tolerance to commensal microorganisms and immunity to pathogens.[5] Considering this complex bacteria–immunity interaction, allogeneic hematopoietic stem cell transplantation (allo-HSCT) has been shown to induce profound gut microbiota perturbations, which, in turn, are highly relevant for transplant-related clinical outcomes.

Current state of the art

Allo-HSCT has been established as a curative therapy for patients with hematopoietic malignancies, hematological deficiencies and immune disorders. Although this therapy has significantly increased survival prospects for many patients, it poses substantial risk to the patient due to infections through immunocompromising of the host, pre-transplant conditioning-induced organ failure, and graft-versus host disease (GvHD) affecting skin, liver, lung and the intestines. In particular, moderate to severe intestinal GvHD occurring in up to 10% of allo-HSCT patients,[6] conveys a substantial risk for transplant-related mortality (TRM), and several lines of evidence point to a major role of the intestinal microbiota in this process. An impact of the gut microflora on GvHD development has been first described in the 1970's by van Bekkum et al. demonstrating that mice kept under germ-free conditions and undergoing allogeneic bone marrow transplantation showed significantly reduced mortality.[7] In parallel, first clinical studies on antibiotic gut decontamination or laminar-airflow isolation also pointed to superior survival after allo-HSCT;[8] however, subsequent clinical trials produced rather mixed results again highlighting the complexity of microbiota – host interactions in allo-HSCT, that we have recently started to understand in more detail since the development of deep-sequencing techniques to uncover the microbiome.

A major advance in the understanding of the impact of the microbiome on GvHD came from observations that a loss of gut microbial diversity after transplant was significantly associated with worse overall survival after allo-HSCT[9] and increased mortality due to GvHD.[10,11] Prophylactic administration of antibiotics or diet changes, e.g., total parental nutrition[10], in the peri- and early post-transplant setting may account for this diversity loss. In addition, we recently reported that use of broad-spectrum antibiotics to treat febrile neutropenia in allo-HSCT patients significantly increases TRM in

contrast to the use of antibiotics with more narrow antibacterial activity[12]. A similar effect of increased TRM was reported by Weber *et al*.[13] regarding the use of broad-spectrum bacterial decontamination by ciprofloxacin/metronidazol *vs*. restricted, gram-positive bacteria targeting using rifaximin.

The post-transplant decrease in gut microflora diversity was not only observed on the level of a reduction of species abundance in the patients' stool specimens, but concomitantly in a reduction of the bacterial metabolite 3-indoxyl sulfate measured in the patients' urine.[11]

The loss of intestinal diversity observed in allo-HSCT patients and in mouse models of GvHD is generally associated with a loss of *Clostridia* species known to have beneficial effects on the host through fermentation of dietary fibers and the release of short chain fatty acids (SCFA).[14] These monocarboxyl acids, especially butyrate, are an important energy source for the epithelium, but also regulate innate and adaptive immune responses, especially regulatory T cells.[15]In line with a *Clostridia* loss, allo-HSCT in mice is associated with a significant reduction of intraepithelial butyrate in the intestinal mucosa.[15] Oral butyrate supplementation or administration of 17 rationally selected strains of high butyrate – producing *Clostridia* to mice subjected to allo-HSCT significantly reduced GvHD-related mortality and enhanced epithelial integrity.[15]

On the level of gut microbiome changes in humans after allo-HSCT, we observed a loss of *Clostridia* and *Bacteroides* species[12] and, notably, the genus *Blautia*[10] that was associated with increased GvHD and TRM. In addition, Proteobacteria, *Lactobacillales*, *Streptococcus* and *Enterococcus* species dominate the post-transplant intestinal flora of allo-HSCT patients, and can lead to severe blood stream infections.[11,14,16]

In addition to the outgrowth of facultative pathogenic bacteria, microbiota perturbances in allo-HSCT also significantly elevate the risk for *Clostridium difficile* infections (CDI) as another clinically relevant post-transplant condition and co-

Table 1. Overview of clinical trials on microbiota manipulation in allo-HSCT setting.

Study title	Study type	Treatment arms	Primary outcomes	Secondary outcomes	Sponsor
Gut decontamination in pediatric allogenic hematopoietic stem cell transplant patients (NCT02641236)	interventional, phase 2; randomized	No gut decontamination vs. vancomycin-polymyxin B	Gut microbiome changes (2 weeks post HSCT)	Incidence of acute GvHD; survival	Dana-Farber te Cancer Institu
Fecal microbiota transplantation after HSCT (NCT02733744)	Interventional, Phase 0; single group assignment	FMT of fecal microbiota in capsules for 15 days	Feasibility of delivery	Incidence of acute GvHD; survival	Massachusetts General Hospital
Autologous fecal microbiota transplantation (Auto-FMT) for prophylaxis of Clostridium difficile infection in recipients of allogeneic hematopoietic stem cell transplantation (NCT02269150)	Interventional, phase 2; randomized	FMT with pre-transplant feces in patients with low post-transplant microbiota diversity vs. standard care	Incidence of CDI	Incidence of acute GvHD; microbiome changes	Memorial Sloan Kettering Cancer Center
Lactobacillus rhamnosus GG in reducing incidence of graft-versus-host disease in patients who have undergone donor stem cell transplant (PERFECT trial) (NCT02144701)	Interventional, phase 2; randomized	No treatment vs. Lactobacillus rhamnosus GG daily for 1 year	Incidence of acute GvHD	Gut microbiome changes; inflammation markers	Rutgers Cancer Institute, NCI
Modification of the intestinal microbiome by diet intervention to mitigate acute graft-versus-host disease (NCT02763033)	Interventional, phase 2; randomized	Standard BMT diet vs. potato-based resistant starch diet (Bob's Red Mill®)	Incidence of acute GvHD	Gut microbiome changes; fecal butyrate; inflammation markers	University of Michigan Cancer Center

Summary compiled according to clinicaltrials.gov.

factor for subsequent development of intestinal GvHD.[17] Fecal microbiota transfer (FMT) can be used to restore a disrupted intestinal flora as proven in recurrent CDI or CDI in allo-HSCT patients.[18] In addition, Kakihana et al. performed a small FMT series patients with steroid-refractory intestinal GvHD and found complete remission of gastrointestinal GvHD symptoms in 3 out of 4 subjects.[19]

Future perspectives

There is a growing understanding of the profound impact of the intestinal microbiota on allo-HSCT patients. As loss of bacterial diversity and shifts in the microbiome profoundly influence morbidity and mortality in allo-HSCT patients the development of novel strategies to monitor and modulate the microbiome are required (see Table 1, summarizing clinical trials on microbiome interventions in allo-HSCT). These include antibiotic regimens with narrow-spectrum antibiotics (e.g., rifaximin[13]), dietary interventions including prebiotics and probiotics,[18] and postbiotics providing bacterial metabolic products as fecal filtrate transfers (FFTs), a novel, experimental approach recently introduced in the treatment of CDI.[20] However, all these microbiological interventions, especially choosing the right antibiotic regimen, require careful clinical decisions given that we are treating individual patients with different risks for infectious complications.

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