Inflammation Research (2021) 70:1–5
https://doi.org/10.1007/s00011-020-01419-w

Inflammation Research

COMMENTARY

Microbiome: an emerging new frontier in graft-versus-host disease

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Received: 11 May 2020 / Revised: 6 October 2020 / Accepted: 26 October 2020 / Published online: 13 November 2020

The field around the microbiome and graft-versus-host disease (GVHD) is rapidly evolving and so is its literature. Therefore, we consider it important to provide an update on our recent review "Microbiome: An Emerging New Frontier in Graft-Versus-Host Disease".

Patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT) are subjected to conditioning, a pre-transplant regimen which involves chemotherapy, radiation, or a combination of both. Often antibiotics are administered to "decontaminate the gut" with the idea to reduce the risk of translocation of intraluminal bacteria of the gut into the blood stream [1] and to reduce pathogen-associated molecular pattern (PAMP) and danger-associated molecular pattern (DAMP)-mediated activation of antigen-presenting cells (APCs). Both the conditioning regimen and antibiotics disturb the homeostatic crosstalk between the microbiome and host immune system and cause microbial dysbiosis, which can affect a patient's risk of developing GVHD [2–4].

Various studies have reported decreased bacterial diversity in GVHD patients [2, 3, 5]. Taur et al. observed association of decreased bacterial diversity with overall survival [6]. Peled et al. also showed that lower diversity of intestinal microbiota was associated with a higher risk of mortality in independent cohorts [7]. It has been shown that gut dysbiosis can be regulated through probiotics, specific diets, and even fecal microbiota transplants (FMT) which can help to treat or prevent intestinal dysbiosis and reduce GVHD severity. Manipulating microbiota remains challenging; however, fecal microbiota transplantation has been proven to be a method by which the microbiome can be manipulated, but this may associate with unknown complications [8, 9]. It is shown that the pre- and post-transplant microbiome can affect the susceptibility to acute GVHD (aGVHD) post-transplant and be of microbial species-specific nature [10].

Bacteremia is one of the complications of allo-HCT which comes about as a result of conditioning-induced disruption of mucosal barrier function [11]. Neutrophils are a critical element in the innate immune response to infection, yet they also contribute to the pathogenesis of aGVHD [12, 13], and depleting neutrophils may not be an appropriate option to reduce the risk of GVHD. Early during the transplant course, neutropenia places the patient at higher risk for infection, often requiring preventive or therapeutic antibiotic treatment. However, this can lead to dysbiosis, and it has been shown that the use of broad-spectrum antibiotics during the neutropenic period is associated with a higher incidence of intestinal GVHD due to loss of microbiome diversity and delayed recovery of myeloid-derived suppressor cells [14]. In critically ill patients, the lung microbiome might become enriched with gut-associated microbes due to increased breaching of mucosal layer, as recently demonstrated in patients with sepsis and acute respiratory distress syndrome (ARDS) [15, 16]. The gut microbiome may predict for pulmonary complications and the baseline dominance of Gammaproteobacteria in the gut predicts pulmonary complications post engraftment and overall mortality [17]. However, to this date, no studies have compared gut and lung microbiome following HCT, which may be helpful to understand the link between gut and lung microbiome. Intestinal microbiota can predict the development of aGVHD in addition to directly inducing aGVHD through inflammatory factors and affecting the Treg/Th17 balance [18]. Hülsdünker et al. reported that active and passive immunization

Responsible Editor: John Di Battista.

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against the conserved microbial surface polysaccharide poly-*N*-acetylglucosamine leads to killing of invading bacteria and reduces uncontrolled neutrophil activation, while maintaining commensal intestinal microbial diversity, thereby reducing GVHD. Antimicrobial therapies that can eliminate invading bacteria and reduce neutrophil-mediated damage without affecting the microbial diversity are promising and highly desirable [19]. A recent report suggested that microbiome profiling may be useful as a prognostic tool that could help identify patients at risk of poor immune reconstitution and adverse outcomes, such as aGVHD, severe infection, or death after allogeneic transplantation. In a longitudinal analysis of immunological markers [20], immune reconstitution and gut microbiota composition in children undergoing HSCT in context of clinical outcomes, researchers identified three consistent clusters showing association of specific bacteria with inflammatory markers: (1) patients with higher concentrations of the antimicrobial peptide human beta-defensin 2 (hBD2) and monocytes prior to transplantation and with high abundances of *Lactobacillaceae*, later developed moderate or severe aGVHD and exhibited high mortality, (2) patients showing rapid reconstitution of NK and B cells with high abundances of obligate anaerobes, such as *Ruminococcace*, developed no or mild aGVHD and exhibited low mortality, (3) patients with high abundances of facultative anaerobic bacteria, such as *Enterobacteriaceae*, demonstrated high levels of inflammation with C-reactive protein as a surrogate marker post HSCT. Therefore, microbiome profiling may serve as a tool to reveal and utilize biomarkers guiding precision medicine-driven prognosis and personalized care [20]. Relapse of primary malignancy remains the leading cause of death after allo-HCT [23]. Studies have reported that the bacterial microbiome plays an important role in normal hematopoiesis [24–26] and furthermore modulates the risk for relapse [21].

The virome of the gastrointestinal tract, also considered as a part of the microbiome composition, consists of vast numbers of viruses, bacteriophages, and endogenous retroviruses, serves as an important site for virus–microbiome–host interactions. Although viruses are typically considered as pathogens, recent studies have indicated a relationship between the host and viruses in the gut which may involve both beneficial and detrimental outcomes for the host. Similar to bacteria, these viruses can lead to immune modulation, which may take place both locally and extra-intestinally [22]. The components of the gut virome can modulate host responses during healthy and disease state and may be involved in immune system maturation. This is supported by the ability of pattern recognition receptors (PRRs) expressed both by immune cells and non-immune cells to recognize nucleic acid derived from enteric viruses. Like bacteria, viral components recognizing PRRs may also contribute to immune system maturation [23]. Legoff et al. showed longitudinal characterization of the gut virome in 44 recipients of HSCT using metagenomics and a viral 'bloom' was identified. Their observation indicated a progressive expansion of vertebrate viral infections over time after HSCT and indicated an involvement of picobirnaviruses with early post-transplant GVHD. Overall increased proportion was represented by both the rates of detection and number of sequences of persistent DNA viruses (anelloviruses, herpesviruses, papillomaviruses and polyomaviruses). Patients with enteric GVHD showed an overall increased proportion over time, relative to those without enteric GVHD. This overall increase was accompanied by reduced phage richness. Picobirnaviruses were detected in 40.9% individuals, more frequently before or within a week after transplant than at later time points, and were predictive of occurrence of severe enteric GVHD in a time-dependent Cox proportional-hazards model, [hazard ratio, 2.66; 95% confidence interval (CI) = 1.46–4.86; $P = 0.001$], and correlated with higher fecal levels of two GVHD severity markers, calprotectin and α1-antitrypsin [24].

A study by Zhang et al. reported FMTs conducted by duodenojejunal infusion from two different donors into a 14-year-old boy with severe life-threatening grade-4 gut aGVHD, refractory to corticosteroids and biologic therapies [9]. Authors observed that FMT altered gut bacterial, fungal and viral communities simultaneously in this GVHD patient, who then clinically recovered. However, bacterial, fungal and virus communities responded differently to FMT: Bacterial diversity was gradually restored after each FMT, engraftment of donor-derived fungi occurred instantly after a single FMT and continued up to 4 months, while viral diversity was improved after multiple FMTs but the composition varied substantially over time. In addition, the ecological network of bacteria–fungi interactions in the recipient was enhanced by serial FMTs, with a significant increase in inter-kingdom correlations after each FMT. Authors suggested that future FMT practice should account for the significance of reconstituting gut fungi and viruses, in addition to bacteria [9]. This observation points out the importance of microbes other than bacteria and the interaction among them, suggesting a potential requirement of serial FMT for maintenance and establishment of gut microbiome specifically for bacteria and viruses. The metabolites derived from microbial fermentation of dietary fibers have shown to be impactful on physiological processes like gut and immune homeostasis, energy metabolism, vascular function, and neurological behavior. These effects are mediated by modulation of G protein-coupled receptor (GPCR) signaling [9, 25, 26]. These reports warrant further studies to investigate the mechanism of immune modulation by microbes.

Seekatz et al. analyzed the patients with recurrent *C. difficile* infection (CDI) which were treated with FMT and explored recovery of the microbiota community and

metabolic environment over time and observed sustained increase in short-chain fatty acids (SCFAs) butyrate, acetate and propionate levels, post-FMT, with variable recovery over time for secondary bile acids deoxycholate and lithocholate. Authors further compared the correlation of these metabolite concentrations changes with specific microbial taxa at an individual level. Metabolites increased post-FMT showed association with bacteria belonging to the *Lachnospiraceae*, *Ruminococcaceae*, and unclassified Clostridiales families. Most of the members within these families were positively correlated with microbial metabolites and inversely with primary bile acids [27]. Bile salt signaling has been shown to play an important role in the mechanism involved in survival and persistence of *C. difficile* in the dysbiotic intestinal environment. Conjugated primary bile salts, promote *C. difficile* germination, in contrast, secondary bile acids (products of microbial metabolism), inhibit germination, growth, and toxin activity of *C. difficile* [27, 28].

While, therefore, FMT is a compelling intervention to restore healthy diversity to the intestinal microenvironment after allo-HCT, it currently has no role as standard of care for transplant recipients [29]. Although the FMTs seemed safe and well tolerated, Bluestone et al. suggested the necessity of larger studies to determine FMT safety and efficacy in immunocompromised HSCT recipients [30]. These reports suggest that FMT assists in restoration of the lost bacterial diversity, thereby outlining its potential to treat CDI and to lead to recovery from GVHD. Therefore, there is no doubt about the benefit of FMT in GVHD. However, to bring it into regular clinical practice, its use needs careful consideration due to the complexity of GVHD pathophysiology.

The research on microbiome composition and its association with diseases is advancing rapidly; however, data related to the functional analysis of metagenome to identify functional signatures of the gut microbiome are scarce. Kyoto Encyclopedia of Genes and Genome (KEGG) Orthology Groups (KOs) are molecular functions represented in terms of functional orthologs which is manually defined in the context of KEGG molecular networks. Microbial dysbiosis in gut and other unknown factors may result in changes in composition of microbial functions which have significant contribution in disease. Therefore, integration of the metagenome for functional characters may help to identify disease etiology.

Armour et al. integrated distinct metagenomic datasets to identify functions encoded in the gut microbiome that associate with multiple diseases. Analysis of gut metagenome protein family richness demonstrated that patients diagnosed with Crohn's disease, obesity, type 2 diabetes, or ulcerative colitis, colorectal cancer show differential number of KOs compared to their respective control populations. However, KOs remained similar for liver cirrhosis or rheumatoid arthritis compared to their respective controls.

It is suggested that the functional composition of the microbiome can classify disease status [31] and requires extensive analysis to find out functional differences of microbiota during disease conditions. The total composition of microbial genes and the presence of genes involved in specific metabolic pathways (the metagenome) are relatively stable between healthy individuals [32] and may change during dysbiosis. Identifying the functional capacity of the microbiome varies in association with disease and across diseases in general, in addition to microbial composition analysis, may reveal the disease-related causes as shown earlier [33, 34].

Studies in GVHD research require understanding the complex relationship between genotype and phenotype on a global (genome-wide) scale to identify functional signatures of the gut microbiome associated with disease etiology. Reports have suggested alteration of butyrate-producing bacteria and pathogenic bacteria in patients and loss of diversity after allogeneic transplant that associates with transplant outcome and inflammatory biomarkers. We think that initiation of a broader transcriptomic approach in GVHD research may help in understanding the pathophysiology and responsible microbial phenotypes.

Over the last decade, there have been multiple reports exploring the microbiome alteration in transplant patients, and these are increasing rapidly. However, the research exploring the translation of these finding to treatment are scarce. There is a big gap between the discovery of microbiome alteration after allogeneic transplant and its relevance to clinical practice.

As discussed above, transcriptomic approaches in GVHD research have yet to be rigorously studied or understood.

Summary

Using advancements in technologies and tools, researchers have discovered and defined a crucial role of the microbiome in physiology and pathophysiology of various diseases. Studies have shown direct impact of dysbiosis in health, physiology, and behavior. However, we do not fully understand the complex networking within the microbial community. Research focusing on the functional genomics of the gut microbiome may advance the understanding of the intricacy of microbial interplay among bacteria, fungi, and viruses. These studies will guide understanding of manipulation of the microbiome composition for host benefits. FMTs and antimicrobial drugs have been successful in regulating and manipulating the microbiome and improving health conditions, but scaling up research efforts on these fronts will help us to gain a greater understanding of the complexity of microbial community networking.

Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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