Curr Stem Cell Rep (2015) 1:39–47
DOI 10.1007/s40778-014-0002-0

MICROBIOME AND STEM CELL FUNCTION (KE NELSON AND MB JONES, SECTION EDITORS)

The Microbiome and Graft Versus Host Disease

Nathan Mathewson · Pavan Reddy

Published online: 16 January 2015

Abstract Allogeneic hematopoietic bone marrow transplantation (BMT) is an established and curative treatment for many aggressive hematological malignancies. However, the success of allogeneic BMT is limited by graft versus host disease (GVHD) due to the attack of recipient organs. There is growing evidence that the commensal microbiota is dysregulated following allogeneic BMT. Recent studies have made significant strides in examining the role of the host and donor microbiome on GVHD severity and pathogenesis. In this review, we summarize the current knowledge of the complex roles of the microbiome on GVHD, as well as the role of the metabolome through which it confers its effects.

Keywords Microbiota · GVHD · Metabolome · Pathogen recognition receptors · Antimicrobial peptides · Short-chain fatty acids

Introduction: Graft Versus Host Disease

Allogeneic hematopoietic bone marrow transplantation (BMT) is a curative therapy for many patients who would otherwise succumb to hematological malignant diseases [1•]. Although BMT increases survival of these patients, 40–50 % of recipients experience complications or secondary disease associated with BMT known as graft versus host disease (GVHD) [2]. GVHD is a complex disease that is modified by the extent of the conditioning regimen, degree of human leukocyte antigen (HLA) mismatch, activation of donor cells, and destruction of target tissues [3, 4].

Conditioning of the host with myeloablative therapy results in damage to host tissues. Damaged tissues respond by producing proinflammatory cytokines (TNFα, IL-1β, IL-6), increased expression of adhesion molecules, and chemokines [5–8]. This inflammatory milieu activates host antigen presenting cells (APC) and results in the upregulation of major histocompatibility complex (MHC) antigens and co-stimulatory molecules [3]. In addition, damage conferred on the gastrointestinal tract sets up the milieu for future stimulation of the immune cells by pathogen-associated molecular patterns (PAMPs) and metabolic by-products produced by the microbiome.

We are beginning to understand the impact of the GI microbiome on GVHD. In this review, we will therefore primarily focus on the GI microbiome and its impact on GVHD and not on the microbiota from other mucosal surfaces.

The Microbiome of the GI Tract

The body is colonized by commensals including bacteria, fungi, and viruses. The human GI tract contains trillions of microorganisms, which is estimated to outnumber human cells 10 to 1 [9, 10]. The gut of a human adult is largely dominated by the phyla Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria [11]. While only a small fraction of these microorganisms may be pathogenic, it is now

This article is part of the Topical Collection on *Microbiome and Stem Cell Function*

N. Mathewson · P. Reddy (✉)
Department of Internal Medicine, Division of Hematology and Oncology, Blood and Marrow Transplantation Program, University of Michigan Comprehensive Cancer Center, 3312 CCC, 1500 E. Medical Center Drive, Ann Arbor, MI 48105-1942, USA
e-mail: reddypr@med.umich.edu

N. Mathewson
e-mail: nmathew@med.umich.edu

N. Mathewson
Graduate Program in Immunology, University of Michigan Medical School, Ann Arbor, MI, USA

appreciated that the relationship between the host and the commensal microbiome as a whole impacts several aspects of the host biology [12, 13•]. Microbiota-associated molecular patterns are directly recognized by pathogen recognition receptors (PRRs). In addition, they secrete a multitude of metabolites that also affect host immunity and biology.

Pathogen Recognition Receptors

Innate immune cells of the host express certain PRRs encoded in the germ-line to detect pathogen-associated molecular patterns (PAMPs) associated with microbes [14]. Recognition of PAMPs through PRRs results in the activation of innate immune cells, such as neutrophils and APCs. In addition to cells of hematopoietic origin, such as the innate and adaptive immune system, PRRs are also expressed on epithelial and endothelial cells.

Several categories of PRRs exist to recognize PAMPs in various cellular surfaces and compartments. One type of PRR is known as toll-like receptors (TLRs). Many TLRs have been described and are known to recognize various PAMPs. These include TLR4 recognition of lipopolysaccharide (LPS), TLR2 of bacterial lipoproteins, TLR5 of flagellin, TLR3 and 7 of RNA, and TLR9 of DNA [15]. The engagement of extracellular TLR receptors requires the adaptor protein known as myeloid differentiation primary response protein 88 (MYD88) for downstream signaling [16]. TLRs have a vast array of functions; however, for the purpose of this review, below, we will focus on those known for affecting the pathogenesis of GVHD. For an in-depth description of general TLRs, we would refer the reader to several comprehensive reviews focused on PRRs [16, 17].

While TLRs are both extracellular and intracellular, another type of PRR, known as NOD-like receptors (NLR), is found in the intracellular compartment [18]. Indeed, NLRs function through the recognition of intracellular PAMPs and danger-associated molecular patterns (DAMP) [19]. NOD1 and NOD2 are the most well studied members of the NLR family and are often viewed as the prototypical NLRs. NOD1 is ubiquitously expressed, where NOD2 expression is restricted to innate immune cells and intestinal Paneth cells [18]. Signaling through the NLR has differential functional effects dependent upon which class of NLR is stimulated. NLR signaling is critical for the function of the inflammasome, a multiprotein complex that plays an important role in inflammatory responses. Activation of NOD1 and NOD2 triggers MAPK and NF-κB pathways, where activated NLRP1, NLRP3, and NLRC4 act as scaffolding platforms for the formation of inflammasomes. A commonality of these three inflammasomes is their association with the protein apoptosis-associated speck-like protein containing a CARD (ASC), which enables the recruitment of caspase-1. The activation of caspase-1 by the inflammasome is required for the processing of IL-1β and IL-18. Although NLRs and TLRs have disparate cellular locations, these PRRs can have both non-redundant and complimentary functions. For example, when cells have become refractory to TLR agonists, NOD1/2 signaling is not mitigated highlighting the non-redundant roles for these PRRs. However, TLR-mediated NF-κB is required for the production of pro-IL-1β, where NLR activation of caspase-1 is required for the cleavage of pro-IL-1β to active IL-1β, leading to its secretion.

Sialic acid-binding Ig-like lectins (Siglecs) are yet another type of PRR. Siglecs, in contrast to other PRRs, are largely inhibitory receptors expressed by neutrophils, monocytes, NK cells, eosinophils, and basophils. Most Siglecs contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) which enable the attenuation of DAMP-mediated inflammation. The ligation of Siglecs by DAMPs reduces NF-κB activation and prevents uncontrolled inflammation in the context of tissue damage [20].

The Metabolome

The microbiota are known to perform key metabolic functions [21]. The microbiota of the gut can metabolize not only material directly ingested by the host but also produce by-products of its own metabolism. The intestinal metabolome thus consists of products from discrete host metabolism, microbial metabolism, and mammalian-microbial co-metabolism [22]. The critical impact of microbiota-derived metabolites is being increasingly appreciated. Donohoe et al. demonstrates that the microbiota plays a critical effect on the energy homeostasis of colonocytes through the generation of short-chain fatty acids (SCFAs). Recent studies show that colonocytes from germ-free mice are in an energy-deprived state demonstrating a decreased ratio of $NADH/NAD^+$, oxidative phosphorylation, and levels of ATP which in turn resulted in autophagy [23].

In addition to the effects of microbial metabolites on non-immune cells, the impact of the metabolome on immune cells is now increasingly being understood. Recent studies have shown that 17 rationally selected strains of Clostridia, known to produce the SCFA butyrate, directly result in the increased presence of regulatory T cells (Tregs) in the gut. Tregs play a critical role in maintaining gastrointestinal homeostasis by modulating inflammatory responses via the release of the anti-inflammatory molecule IL-10, which also directly impact macrophages [24–27]. Similarly, other groups have utilized a cocktail of altered Schaedler flora which too resulted in the de novo generation of colonic Tregs [28].

With the emerging importance of the effects of microbial metabolites on host biology beginning to be appreciated, recent studies have shown diet to play a role in regulating rapid changes in the taxonomic composition of the gut microbiome [29, 30]. These findings suggest that an

appropriate diet conducive to homeostatic microbiota may be an important factor when treating comorbidities as the associated metabolome is correspondingly altered [31, 32].

Together, these data suggest the microbial metabolome could impact GVHD, although this hypothesis remains to be formally tested.

Antimicrobial Peptides

It has long been established that colonization of commensal microflora provides protection against invading pathogenic bacteria, referred to as colonization resistance [33]. Further, intestinal epithelial cells (IECs) provide a physical barrier against contaminating factors found in the lumen of the gut (Fig. 1). IECs are known to produce both antimicrobial peptides (AMPs) which inhibit the growth of and kill microorganisms, and a polysaccharide-rich mucus layer which functions in a manner similar to biofilms [34]. The biofilm-like mucus promotes various functions of the microbiota including metabolism of luminal contents, fortification of host defenses, and resistance to hydrodynamic forces due to peristalsis [34].

Defensins and cathelicidins are AMPs that have been identified in many mammals. Defensins interact with, and potentially destroy, both Gram-negative and Gram-positive bacteria through membrane disruption [35, 36]. Several studies have also described the ability of defensins to sequester components of the bacterial cell wall, thus inhibiting its synthesis [35, 37]. These findings emphasize the ability of defensins to mount a multifaceted attack on bacterial targets.

Recent studies have demonstrated the rapid release of defensins not only neutralizes pathogenic bacteria, but they are also sufficient to initiate and amplify an adaptive immune response resulting in both Th1-dependant cellular responses and Th2-dependent humoral responses by activation of immature dendritic cells (DC) [38].

Cathelicidin (LL-37) is chiefly produced and stored in granules of neutrophils; however, it is also an inducible product of epithelial cells, T cells, and monocytes [39].

Another type of AMP, RegIIIα (RegIIIγ in mice), is a C-type lectin, found primarily in the intestine, and is generated by Paneth cells. RegIII is composed of a combination of α-helical structures and beta sheets [40] and binds to peptidoglycan carbohydrates of Gram-positive bacterial cell walls, in a calcium independent process.

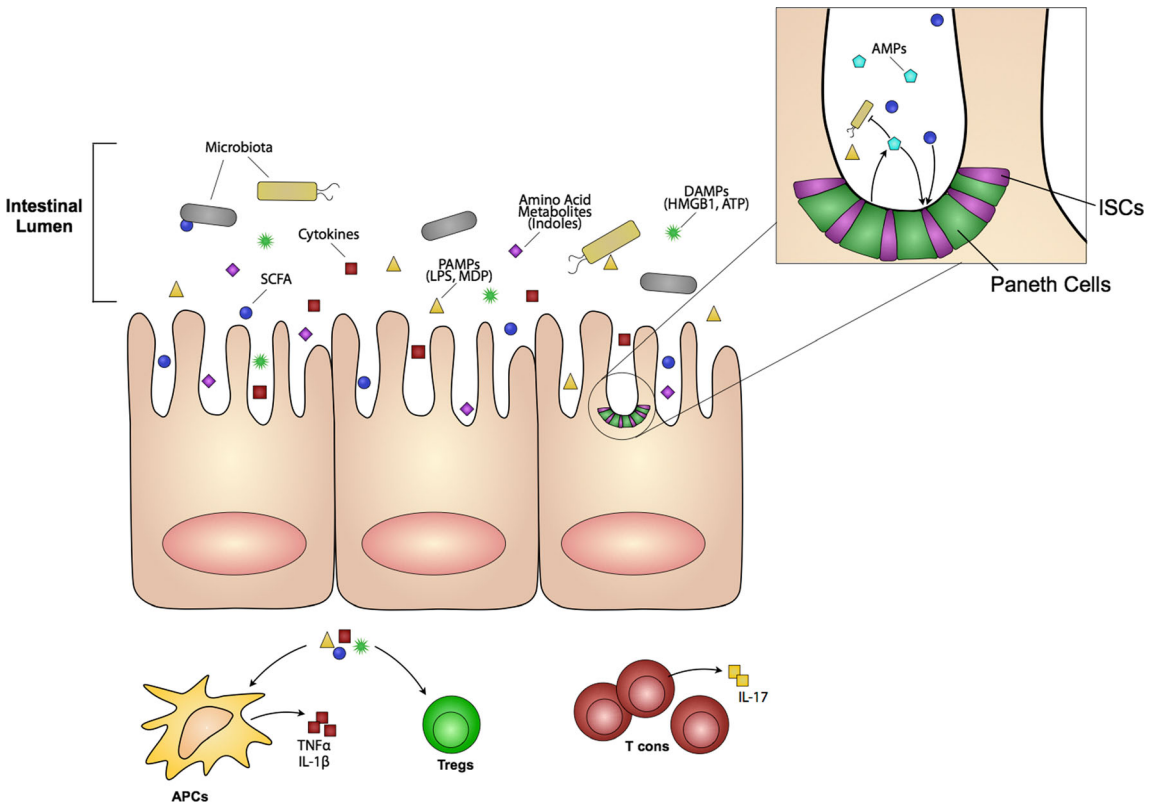

Fig. 1 The intestinal barrier and gut homeostasis. The intestinal lumen contains microbial by-products particularly SCFAs, PAMPs, and other metabolites such as indoles. Host intestinal epithelial cells (IECs) provide a physical barrier against contaminating PAMPs and produce cytokines and DAMPs that are found in the lumen. Specialized IECs, known as Paneth cells, produce antimicrobial peptides (AMPs) that selectively inhibit pathogenic bacteria while preserving commensal microbiota and providing critical trophic factors for intestinal stem cells. Damage to IEC homeostasis results in loss of barrier function and activation of immune cells

Studies have shown that RegIIIα adopts a hexameric membrane-permeating pore structure to kill bacteria [41, 42].

In addition to AMPs, IECs also secrete chemotactic cytokines (chemokines) resulting in the recruitment of innate immune cells (Table 1) and produce proinflammatory enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) which activate neutrophils resulting in their degranulation upon pathogenic stimuli [43].

Thus, in coordination with immune cells, IECs can have a significant influence on both the microbiota and homeostasis of the host tissue.

The Role of the Microbiome in GVHD

Early Experimental Studies

A role for the microbiome in modulating the severity of GVHD was first identified in the early 1960s by the seminal studies of van Bekkum et al. using germ-free recipient mice. The authors made the observation that the severity of GVHD was markedly decreased when compared to conventional BMT controls [44, 45]. Subsequent studies by the same and other groups confirmed and expanded these findings [46, 47]. Further studies by van Bekkum et al. isolated colonization resistant microflora which inhibited colonization of *Escherichia coli*, *K. pneumoniae*, and *P. aeruginosa* by treating conventionally housed mice with antibiotics (streptomycin, neomycin, and pimaricin) [48]. The colonization resistant microflora were then transferred to germ-free mice prior to BMT resulting in decreased GVHD severity, thus indicating that select microorganisms may be beneficial in the context of BMT. These studies formed the basis for clinical utilization of antibiotic prophylaxis prior to BMT and the establishment of standard practice gut decontamination prior to BMT in the clinic, at many transplants centers.

Table 1 Chemokines secreted by IECs

Chemokine	Cell type attracted	Function
Human: IL-8; CXCL8 ENA-78; CXCL5 Gro-α; CXCL1 Gro-β; CXCL2 Murine: KC	Neutrophils	Regulation of chemotaxis. IL-8 is released faster than the longer acting ENA-78.
Human: MCP-1; CCL2 MIP1α; CCL3 RANTES; CCL5	Monocytes	Regulate monocyte recruitment. MIP1α plays a major role in recruiting mucosal DCs.
Human: IP-10 Mig I-TAC	T cells	Constitutively expressed. Consistent with the fact that intraepithelial lymphocytes (IEL) are normally present in the mucosa.

Clinical Studies

The early experimental studies described above led to initial clinical trials designed to determine the role of GI bacterial decontamination in BMT patients. In a study performed nearly 30 years ago, patients were divided into 3 groups: administration of oral nonabsorbable antibiotics with isolation and decontamination in laminar airflow isolation (LAF) rooms, prophylactic granulocyte transfusions from a single family member donor, or conventional treatment in single rooms with hand-washing and mask precautions [49]. Following engraftment, significantly fewer infections were observed in patients isolated in LAF rooms and acute GVHD occurred much later than control groups. More importantly, day 100 overall survival was significantly improved in patients in LAF isolation (92 %) compared to groups in conventional treatment (64 %) [49]. Another study in which patients were either treated with meropenem, a broad-spectrum antibiotic, starting on the first day of febrile episode, or prophylactically treated beginning the first day with $<500/mm^3$ granulocytes, demonstrated fewer febrile episodes in patients receiving meropenem prophylaxis. Prophylactic use of meropenem during the period of neutropenia favorably affected the morbidity of the BMT procedure suggesting that reduced febrile episodes were due to decreased bacterial infections [50].

Other studies indicate that increased survival in patients treated in LAF rooms resulted in less transplant-related mortality (TRM) independent of prophylactic antibiotic use [51], whereas other studies indicated that there is no benefit to isolation in LAF room following BMT [52]. Several groups have performed additional studies utilizing antibiotic prophylaxis prior to BMT or during granulocytopenic period following BMT with conflicting results. One such study housed all patients in LAF rooms with one group receiving prophylactic systemic antibiotics (PSA). However, overall survival at day 100 of groups in LAF rooms with PSA was 72.2 % where groups in LAF rooms only was 70.6 % suggesting no improvement in survival, even though LAF + PSA groups had significantly lower incidence of infections [53]. However, many of these studies have sought to answer disparate questions and lacked sufficient power to determine the validity of prophylactic antibacterial use on GVHD severity in the patients.

The Impact of the Microbiome of the Host on GVHD: Recent Observations

Despite early observations as noted above, the role that the host microbiome plays in the severity of GVHD was largely unexplored until recent years. Recent studies have made

significant strides in illuminating alterations in the intestinal microbiota following allogeneic BMT in mice and humans [54••, 55]. Jenq et al. described observations utilizing clinical models of BMT (B10.BR→B6) where recipients were subjected to lethal irradiation and received donor BM with or without isolated T cells. A loss of overall diversity was seen consisting of an expansion of Lactobacillales with a simultaneous loss of Clostridiales in allo-recipients of BM with isolated T cells. Elegant experiments where the predominant pre-BMT species of *Lactobacillus* (*L. johnsonii*) was reintroduced following gut decontamination and allo-BMT, resulted in improved overall survival compared to recipients of allo-BMT receiving only gut decontamination [54••]. Likewise, unpublished observations from our laboratory suggest that intragastric introduction of a cocktail of 17 rationally selected strains of predominantly Clostridiales [24] with salutary effects on the GI epithelium, results in decreased GVHD and improved overall survival [56].

Another recent study illuminated the impact of the loss of diversity in the gut further, following allo-BMT. The study observed that Paneth cells are targets of GVHD [55]. Paneth cells, located next to intestinal stem cells (ISC) within the crypts of the intestinal lumen (Fig. 1), are essential regulators of the composition of the intestinal microbiota [57, 58]. Further, Paneth cells are known to secrete antimicrobial peptides and α-defensins which largely function by forming pores in bacterial cell walls [59, 60]. Eriguchi et al. revealed that the loss of Paneth cells from GVHD resulted in decreased expression of α-defensins. The authors further suggest that α-defensins selectively kill non-commensal bacteria while preserving commensal microbiota [55]. The study suggests that the decrease in the expression of α-defensins resulted in the loss of microbial diversity (as also observed in Jenq et al.) and an overwhelming expansion of *E. coli*, leading to septicemia.

A recent study demonstrated the importance of bacterial diversity in the intestinal tract on mortality outcomes following BMT. The authors collected fecal specimens from patients that received allo-BMT, at the time of stem cell engraftment [61]. Microbial diversity was then determined by performing bacterial 16S rRNA gene sequencing. Patients with lower bacterial diversity exhibited significantly worse mortality. Overall survival at 3 years was 36 % for low, 60 % for intermediate, and 67 % for high diversity groups [61]. These results suggest that diversity of the intestinal microbiota at time of engraftment may be a predictor of mortality in recipients of allo-BMT and thus highlight the consequences of dysbiosis of the intestinal flora.

The Impact of the Microbiome of the Donor

The microbiome is known to play a key role in the development and maturation of T cells [24, 62, 63]. The influence of the donor microbiota on the donor allograft and its impact on GVHD was recently examined [64]. Using clinically relevant murine models of BMT, the authors observed the severity of GVHD induced by T cells harvested from germ-free (GF) donors and specific pathogen-free (SPF) donors. Interestingly, there was no difference in the frequency of Treg cells in the periphery of the host or levels of lineage-specific cytokines in the sera such as IFNγ, IL-5, IL-17, or the anti-inflammatory cytokine IL-10. Further, clinical observation revealed that GVHD progression and severity was similar between recipients of the GF and SPF donor T cells with no difference in histopathological scores of the small intestine and liver. In addition, no survival benefit was observed in the recipients of donor T cells from GF or SPF donor T cells. Furthermore, when SPF donor mice were treated with broad-spectrum antibiotics prior to T cell isolation, no difference in survival was determined.

These data indicate that absence of the effects of the microbiome on T cells may be lost once they have been removed from the germ-free environment.

PAMPs and GVHD

There is mounting evidence that microorganisms and PRRs of the innate immune system play a critical role in the pathogenesis of acute GVHD [45, 54••, 65]. Products of Gram-negative bacteria, such as LPS, were suggested to be contributors to the severity and progression of GVHD in certain experimental models [66–68]. Cooke et al. utilized a potent antagonist of LPS and observed significantly reduced production of TNFα and intestinal damage. While donor T cell responses were unaltered, overall GVHD severity was reduced [67]. However, a recent study utilized a different model system with myeloid differentiation primary response gene (88) (MYD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF) double knockout cells, thus the donor allograft was deficient in all TLR signaling [69]. The authors demonstrated that loss of TLR signaling did not protect from GVHD nor improve overall survival, suggesting that TLR signaling is dispensable for GVHD severity.

Human genetic association studies wherein patients possessing TLR4 polymorphisms, who also received grafts from HLA-matched donor siblings, demonstrated an increased risk for Gram-negative bacteremia and GVHD severity [70].

In another study, TLR9 deficient recipients of allo-BMT have demonstrated decreased systemic GVHD [71]. The authors observed that APCs isolated from $TLR9^{-/-}$ mice had a decreased ability to stimulate allogeneic T cells. Further, utilizing cytosine-phosphorothioate-guanine oligodeoxynucleotides (CpG ODNs), which mimics bacterial and viral DNA and stimulates TLR9, Blazar et al. found that CpG treatment enhanced allo-T cell responses leading to increased GVHD severity and

mortality [72]. These TLR9 dependent studies emphasize the importance of TLR signaling for host APC function during GVHD [73]. The analysis of TLR9 gene associated polymorphisms on the clinical outcome of 413 donors showed no association of the TLR9 polymorphisms with incidence or severity of GVHD [70, 73]. However, patients with the T1486C mutation exhibited significantly improved survival due to reduced TRM and relapse rate.

The importance of TLRs in neutrophils on the severity of GVHD has recently been demonstrated. Neutrophils are the largest human white blood cell population and have important roles in cleaving chemokines and the production of reactive oxygen species (ROS). During allogeneic immune responses, neutrophils amplify tissue damage caused by conditioning regimens [74]. Schwab et al. utilized clinical models of BMT where donor neutrophils were TLR2, TLR3, TLR4, TLR7, and TLR9 deficient. GVHD severity was significantly reduced suggesting that TLR signaling is important for neutrophil-mediated inflammation in the context of allo-BMT.

The role of NLRs in GVHD has been examined in several recent studies. NOD2 deficiency in recipients of experimental BMT resulted in increased GVHD of both MHC-mismatched and MHC-matched models [75, 76] corresponding with enhanced activation and proliferation of donor T cells, due to increased activation status of DCs.

The presence of certain cytokines, such as IL-1β, was suggested to predict the outcome and severity of GVHD [6]. IL-1β is one such proinflammatory cytokine that is released following NLR stimulation. A study examining the genotypes of 133 patients undergoing BMT from (HLA)-matched donor siblings demonstrated a strong correlation between genetic variants of NLRP2 and NLRP3 genes and the clinical outcome and severity of GVHD [77].

Immune-mediated tissue destruction, which is often found in acute GVHD, is a result of cellular damage or response to DAMPs. Although critical for the function of the cell, extracellular ATP released from a damaged cell is subsequently internalized and serves as a strong activating signal of NLRP3 [78]. In response to accumulated ATP and NLRP3 stimulation, APCs increased expression of co-stimulatory molecules which resulted in enhanced proinflammatory signals and expansion of donor T cells with a reduction of Treg cells and increased GVHD severity [79].

Siglec-G recognition of non-infectious DAMPs regulates innate immune responses [80]. A recent study examined the role of Siglec-G expression on host APCs, specifically on hematopoietic cells, and its ability to negatively regulate GVHD in multiple clinically relevant murine models [81]. The authors demonstrate that recipients deficient in Siglec-G exhibit significantly increased GVHD severity and mortality, in a CD24 dependent manner. Upon administration of CD24 fusion protein to WT recipients of allo-BMT, improved overall survival is observed. However, administration of CD24 fusion protein to recipients deficient in Siglec-G did not improve survival, suggesting that Siglec signaling is required for the effects of CD24 [81].

These data suggest that the Siglec-G–CD24 axis controls the severity of GVHD and that enhancing this interaction may represent a method of mitigating clinical GVHD.

The Metabolome and GVHD

Many recent studies have made strides to identify the taxonomic composition and activity of the host microbiome [24, 32, 54••]. However, few publications examine the role of microbial metabolites (e.g., SCFAs) in the homeostasis of host physiology. While it is increasingly evident that alterations in the microbiome correlate with many disease states [82–84], the mechanism through which these alterations confer their effects is poorly understood.

The most studied microbial metabolites are SCFAs, of which butyrate, acetate, and propionate are the most abundant. As described earlier in this review, SCFAs are produced by microbial fermentation of complex polysaccharides in the gut and are subsequently absorbed by the intestinal epithelium [21]. Butyrate is utilized as a major energy source for IECs and is a known histone deacetylase inhibitor (iHDAC) [85]. Thus, microbial metabolites may play an important role in maintaining the health of the physical barrier of the gut epithelium as well as protecting the host from the influences of PAMPs and DAMPs.

SCFAs may have a particularly important role in the protection of the host from GVHD. Unpublished observations from our lab demonstrate that there is a significantly decreased concentration of butyrate found in the tissue of the GI tract following allo-BMT. In addition, this coincides with a substantial and significant decrease in the acetylation state of histones in IECs of allo-BMT recipients. When butyrate was supplemented via intragastric gavage, it increased histone acetylation in IECs and significantly improved junction integrity mitigating GVHD severity [56].

AMPs and GVHD

AMPs, produced by Paneth cells, have been shown to be a critical "first line of defense" of the innate immune system among epithelial barriers [86, 87]. As discussed above, a recent study observed a loss of α-defensins in the gut due to a GVHD-mediated decrease of Paneth cells [55]. Furthermore, non-commensal bacteria were selectively targeted and killed by α-defensins, while commensal microbiota were preserved. Equally, several studies have observed that the composition of the intestinal microbiome is directly influenced by alterations in the expression of AMPs [88, 89].

Another recent study by Ferrara et al. identified the AMP RegIIIα as a biomarker found in the plasma indicative of acute

GVHD in the lower GI tract. The authors found RegIIIα to be 3-fold higher at GI GVHD onset in a large cohort of patients, compared to control cohorts [90].

Conclusions

The effects of the microbiome on GVHD, and that of GVHD on the microbiome, are now being increasingly appreciated. The differential role that the microbiota plays in the host [54••, 55] and the donor [64] and the effects of polymorphisms of PRRs on GVHD severity highlight the complex interactions between the microbiome and GVHD that remain to be meticulously examined further. Further studies examining the functions and mechanisms are needed to identify new targets for treating GVHD and other diseases affected by dysbiosis of the microbiota.

Compliance with Ethics Guidelines

Conflict of Interest Nathan Mathewson and Pavan Reddy declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1.• Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. Nat Rev Immunol. 2012;12:443–58. *This review provides a thorough description of GVHD and highlights the role of the immune system in GVHD pathogenesis*.
2. Jacobsohn DA, Vogelsang GB. Acute graft versus host disease. Orphanet J Rare Dis. 2007;2:35.
3. Ferrara JLM, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009;373:1550–61.
4. Paczesny S, Hanauer D, Sun Y, Reddy P. New perspectives on the biology of acute GVHD. Bone Marrow Transplant. 2010;45:1–11.
5. Nestel FP, Price KS, Seemayer TA, Lapp WS. Macrophage priming and lipopolysaccharide-triggered release of tumor necrosis factor alpha during graft-versus-host disease. J Exp Med. 1992;175:405–13.
6. Ferrara JL, Abhyankar S, Gilliland DG. Cytokine storm of graft-versus-host disease: a critical effector role for interleukin-1. Transplant Proc. 1993;25:1216–7.
7. Tawara I, Koyama M, Liu C, Toubai T, Thomas D, Evers R, et al. Interleukin-6 modulates graft-versus-host responses after experimental allogeneic bone marrow transplantation. Clin Cancer Res. 2011;17:77–88.
8. Hill GR, Crawford JM, Cooke KR, Brinson YS, Pan L, Ferrara JL. Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. Blood. 1997;90:3204–13.
9. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005;307:1915–20.
10. Savage DC. Microbial ecology of the gastrointestinal tract. Annu Rev Microbiol. 1977;31:107–33.
11. Nuding S, Antoni L, Stange EF. The host and the flora. Dig Dis. 2013;31:286–92.
12. Khosravi A, Yáñez A, Price JG, Chow A, Merad M, Goodridge HS, et al. Gut microbiota promote hematopoiesis to control bacterial infection. Cell Host Microbe. 2014;15:374–81.
13.• Blaser MJ. The microbiome revolution. J Clin Invest. 2014. *This review provides a thorough overview of the microbiome and its effects at various stages of human development.*
14. Reikine S, Nguyen JB, Modis Y. Pattern recognition and signaling mechanisms of RIG-I and MDA5. Front Immunol. 2014;5:342.
15. Cook DN, Pisetsky DS, Schwartz DA. Toll-like receptors in the pathogenesis of human disease. Nat Immunol. 2004;5:975–9.
16. Gay NJ, Symmons MF, Gangloff M, Bryant CE. Assembly and localization of Toll-like receptor signalling complexes. Nat Rev Immunol. 2014;14:546–58.
17. Abdullah Z, Knolle PA. Scaling of immune responses against intracellular bacterial infection. EMBO J. 2014;33:2283–94.
18. Shaw MH, Reimer T, Kim Y-G, Nuñez G. NOD-like receptors (NLRs): bona fide intracellular microbial sensors. Curr Opin Immunol. 2008;20:377–82.
19. Lechtenberg BC, Mace PD, Riedl SJ. Structural mechanisms in NLR inflammasome signaling. Curr Opin Struct Biol. 2014;29C:17–25.
20. Pillai S, Netravali IA, Cariappa A, Mattoo H. Siglecs and immune regulation. Annu Rev Immunol. 2012;30:357–92.
21. Natarajan N, Pluznick JL. From microbe to man: the role of microbial short chain fatty acid metabolites in host cell biology. Am J Physiol Cell Physiol. 2014. doi:10.1152/ajpcell.00228.2014.
22. Young VB. The intestinal microbiota in health and disease. Curr Opin Gastroenterol. 2012;28:63–9.
23. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metab. 2011;13:517–26.
24. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature. 2013.
25. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013.
26. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol. 2003;3:521–33.
27. Palm NW, Medzhitov R. Not so fast: adaptive suppression of innate immunity. Nat Med. 2007;13:1142–4.
28. Geuking MB, Cahenzli J, Lawson MAE, Ng DCK, Slack E, Hapfelmeier S, et al. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. Immunity. 2011;34:794–806.
29. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med. 2009;1:6ra14.
30. Zivkovic AM, German JB, Lebrilla CB, Mills DA. Human milk glycobiome and its impact on the infant gastrointestinal microbiota. Proc Natl Acad Sci U S A. 2011;108 Suppl 1:4653–8.
31. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505:559–63.
32. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, et al. Microbiota-generated metabolites

promote metabolic benefits via gut-brain neural circuits. Cell. 2014;156:84–96.
33. Stecher B, Hardt W-D. Mechanisms controlling pathogen colonization of the gut. Curr Opin Microbiol. 2011;14:82–91.
34. Sonnenburg JL, Angenent LT, Gordon JI. Getting a grip on things: how do communities of bacterial symbionts become established in our intestine? Nat Immunol. 2004;5:569–73.
35. Chairatana P, Nolan EM. Molecular basis for self-assembly of a human host-defense peptide that entraps bacterial pathogens. J Am Chem Soc. 2014;136:13267–76.
36. Yount NY, Yeaman MR. Peptide antimicrobials: cell wall as a bacterial target. Ann N Y Acad Sci. 2013;1277:127–38.
37. Zhao L, Lu W. Defensins in innate immunity. Curr Opin Hematol. 2014;21:37–42.
38. Ricciardi-Castagnoli P, Granucci F. Opinion: Interpretation of the complexity of innate immune responses by functional genomics. Nat Rev Immunol. 2002;2:881–9.
39. Oppenheim JJ, Biragyn A, Kwak LW, Yang D. Roles of antimicrobial peptides such as defensins in innate and adaptive immunity. Ann Rheum Dis. 2003;62 Suppl 2:ii17–21.
40. Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science. 2006;313:1126–30.
41. Mukherjee S, Zheng H, Derebe MG, Callenberg KM. Antibacterial membrane attack by a pore-forming intestinal C-type lectin. Nature. 2013.
42. Wang G. Pharmaceuticals | free full-text | human antimicrobial peptides and proteins | HTML. Pharmaceuticals. 2014.
43. Kolios G, Valatas V, Ward SG. Nitric oxide in inflammatory bowel disease: a universal messenger in an unsolved puzzle. Immunology. 2004;113:427–37.
44. van BEKKUM D, VOS O. Treatment of secondary disease in radiation chimaeras. Int J Radiat Biol. 1961;3:173–81.
45. van Bekkum DW, De Vries MJ, van der Waay D. Lesions characteristic of secondary disease in germfree heterologous radiation chimeras. 1967.
46. Jones JM, Wilson R, Bealmear PM. Mortality and gross pathology of secondary disease in germfree mouse radiation chimeras. Radiat Res. 1971;45:577–88.
47. van Bekkum DW, Roodenburg J, Heidt PJ, van der Waaij D. Mitigation of secondary disease of allogeneic mouse radiation chimeras by modification of the intestinal microflora. J Natl Cancer Inst. 1974;52:401–4.
48. van der Waaij D, Berghuis-de Vries JM, Lekkerkerk Lekkerkerk-v. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. J Hyg (Lond). 1971;69:405–11.
49. Navari RM, Buckner CD, Clift RA, Storb R, Sanders JE, Stewart P, et al. Prophylaxis of infection in patients with aplastic anemia receiving allogeneic marrow transplants. Am J Med. 1984;76:564–72.
50. Pérez-Simón JA, García-Escobar I, Martinez J, Vazquez L, Caballero D, Cañizo C, et al. Antibiotic prophylaxis with meropenem after allogeneic stem cell transplantation. Bone Marrow Transplant. 2004;33:183–7.
51. Passweg JR, Rowlings PA, Atkinson KA, Barrett AJ, Gale RP, Gratwohl A, et al. Influence of protective isolation on outcome of allogeneic bone marrow transplantation for leukemia. Bone Marrow Transplant. 1998;21:1231–8.
52. Russell JA, Chaudhry A, Booth K, Brown C, Woodman RC, Valentine K, et al. Early outcomes after allogeneic stem cell transplantation for leukemia and myelodysplasia without protective isolation: a 10-year experience. Biol Blood Marrow Transplant. 2000;6:109–14.
53. Petersen FB, Buckner CD, Clift RA, Nelson N, Counts GW, Meyers JD, et al. Infectious complications in patients undergoing marrow transplantation: a prospective randomized study of the additional effect of decontamination and laminar air flow isolation among patients receiving prophylactic systemic antibiotics. J Infect Informa UK Ltd UK. 1987.
54. •• Jenq RR, Ubeda C, Taur Y, Menezes CC, Khanin R, Dudakov JA, et al. Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. J Exp Med. 2012;209:903–11. *This study describes taxonomic alterations in the microbiota that occur following allogeneic bone marrow transplantation.*
55. Eriguchi Y, Takashima S, Oka H, Shimoji S, Nakamura K, Uryu H, et al. Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of α-defensins. Blood. 2012;120:223–31.
56. Mathewson N, Mathew A, Oravecz-Wilson K, Wu J, Toubai T, Rossi C, et al. Unbiased metabolic profiling uncovers a crucial role for the microbial metabolite butyrate in modulating GI epithelial cell damage from Gvhd [Internet]. Am Soc Hematol. 2014 [cited 2014 Nov 10]. Available from: https://ash.confex.com/ash/2014/webprogram/Paper75934.html
57. Vermeulen L, Snippert HJ. Stem cell dynamics in homeostasis and cancer of the intestine. Nat Rev Cancer. 2014;14:468–80.
58. Sato T, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, et al. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature. 2011;469:415–8.
59. Selsted ME, Harwig SS. Determination of the disulfide array in the human defensin HNP-2. A covalently cyclized peptide. J Biol Chem. 1989;264:4003–7.
60. Bevins CL. Innate immune functions of α-defensins in the small intestine. Dig Dis. 2013;31:299–304.
61. Taur Y, Jenq RR, Perales M-A, Littmann ER, Morjaria S, Ling L, et al. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. Blood. 2014;124:1174–82.
62. Bleich A, Janus LM, Smoczek A, Westendorf AM, Strauch U, Mähler M, et al. CpG motifs of bacterial DNA exert protective effects in mouse models of IBD by antigen-independent tolerance induction. Gastroenterology. 2009;136:278–87.
63. Furusawa Y, Obata Y, Hase K. Commensal microbiota regulates T cell fate decision in the gut. Semin Immunopathol. 2014.
64. Tawara I, Liu C, Tamaki H, Toubai T, Sun Y, Evers R, et al. Influence of donor microbiota on the severity of experimental graft-versus-host-disease. Biol Blood Marrow Transplant. 2013;19:164–8.
65. Heidegger S, van den Brink MRM, Haas T, Poeck H. The role of pattern-recognition receptors in graft-versus-host disease and graft-versus-leukemia after allogeneic stem cell transplantation. Front Immunol. 2014;5:337.
66. Bayston K, Baumgartner JD, Clark P, Cohen J. Anti-endotoxin antibody for prevention of acute GVHD. Bone Marrow Transplant. 1991;8:426–7.
67. Cooke KR, Gerbitz A, Crawford JM, Teshima T, Hill GR, Tesolin A, et al. LPS antagonism reduces graft-versus-host disease and preserves graft-versus-leukemia activity after experimental bone marrow transplantation. J Clin Invest. 2001;107:1581–9.
68. Cohen J, Moore RH, Hashimi Al S, Jones L, Apperley JF, Aber VR. Antibody titres to a rough-mutant strain of Escherichia coli in patients undergoing allogeneic bone-marrow transplantation. Evidence of a protective effect against graft-versus-host disease. Lancet. 1987;1:8–11.
69. Li H, Matte-Martone C, Tan HS, Venkatesan S, McNiff J, Demetris AJ, et al. Graft-versus-host disease is independent of innate signaling pathways triggered by pathogens in host hematopoietic cells. J Immunol. 2011;186:230–41.
70. Elmaagacli AH, Koldehoff M, Hindahl H, Steckel NK, Trenschel R, Peceny R, et al. Mutations in innate immune system NOD2/CARD 15 and TLR-4 (Thr399Ile) genes influence the risk for severe acute graft-versus-host disease in

patients who underwent an allogeneic transplantation. Transplantation. 2006;81:247–54.
71. Calcaterra C, Sfondrini L, Rossini A, Sommariva M, Rumio C, Ménard S, et al. Critical role of TLR9 in acute graft-versus-host disease. J Immunol. 2008;181:6132–9.
72. Taylor PA, Ehrhardt MJ, Lees CJ, Panoskaltsis-Mortari A, Krieg AM, Sharpe AH, et al. TLR agonists regulate alloresponses and uncover a critical role for donor APCs in allogeneic bone marrow rejection. Blood. 2008;112:3508–16.
73. Penack O, Holler E, van den Brink MRM. Graft-versus-host disease: regulation by microbe-associated molecules and innate immune receptors. Blood. 2010;115:1865–72.
74. Schwab L, Goroncy L, Palaniyandi S, Gautam S, Triantafyllopoulou A, Mocsai A, et al. Neutrophil granulocytes recruited upon translocation of intestinal bacteria enhance graft-versus-host disease via tissue damage. Nat Med. 2014;20:648–54.
75. Penack O, Smith OM, Cunningham-Bussel A, Liu X, Rao U, Yim N, et al. NOD2 regulates hematopoietic cell function during graft-versus-host disease. J Exp Med. 2009;206:2101–10.
76. Shin OS, Harris JB. Innate immunity and transplantation tolerance: the potential role of TLRs/NLRs in GVHD. Korean J Hematol. 2011;46:69–79.
77. Granell M, Urbano-Ispizua A, Pons A, Aróstegui JI, Gel B, Navarro A, et al. Common variants in NLRP2 and NLRP3 genes are strong prognostic factors for the outcome of HLA-identical sibling allogeneic stem cell transplantation. Blood. 2008;112:4337–42.
78. Zeiser R, Penack O, Holler E, Idzko M. Danger signals activating innate immunity in graft-versus-host disease. J Mol Med. 2011;89:833–45.
79. Wilhelm K, Ganesan J, Müller T, Dürr C, Grimm M, Beilhack A, et al. Graft-versus-host disease is enhanced by extracellular ATP activating P2X7R. Nat Med. 2010;16:1434–8.
80. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. Nat Rev Immunol. 2007;7:255–66.
81. Toubai T, Guoqing H, Mathewson N, Liu C, Wang Y, Oravecz-Wilson K, et al. Siglec-G-CD24 axis controls the severity of graft-versus-host disease in mice. Blood. 2014;123:3512–23.
82. Vaziri ND, Yuan J, Norris K. Role of urea in intestinal barrier dysfunction and disruption of epithelial tight junction in chronic kidney disease. Am J Nephrol. 2013;37:1–6.
83. Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J, et al. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. Proc Natl Acad Sci U S A. 2013;110:4410–5.
84. DuPont AW, DuPont HL. The intestinal microbiota and chronic disorders of the gut. Nat Rev Gastroenterol Hepatol. 2011;8:523–31.
85. Wong JMW, de Souza R, Kendall CWC, Emam A, Jenkins DJA. Colonic health: fermentation and short chain fatty acids. J Clin Gastroenterol. 2006;40:235–43.
86. Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, et al. Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature. 2001;414:454–7.
87. Bals R, Weiner DJ, Meegalla RL, Wilson JM. Transfer of a cathelicidin peptide antibiotic gene restores bacterial killing in a cystic fibrosis xenograft model. J Clin Invest. 1999;103:1113–7.
88. Wehkamp J, Salzman NH, Porter E. Reduced Paneth cell α-defensins in ileal Crohn's disease. 2005.
89. Salzman NH. Paneth cell defensins and the regulation of the microbiome: détente at mucosal surfaces. Gut Microbes. 2010;1:401–6.
90. Ferrara JLM, Harris AC, Greenson JK, Braun TM, Holler E, Teshima T, et al. Regenerating islet-derived 3-alpha is a biomarker of gastrointestinal graft-versus-host disease. Blood. 2011;118:6702–8.