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Antibiotic-mediated modification of the intestinal microbiome in allogeneic hematopoietic stem cell transplantation

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Abstract

Allogeneic hematopoietic stem cell transplantation (HSCT) is curative for many patients with severe benign and malignant hematologic disorders. The success of allogeneic HSCT is limited by the development of transplant-related complications such as acute graft-versus-host disease (GvHD). Early pre-clinical studies suggested that intestinal microflora contribute to the pathogenesis of acute GvHD, and that growth suppression or eradication of intestinal bacteria prevented the development of acute GvHD even in MHC-mismatched transplants. These observations led to the practice of gut decontamination (GD) with oral non-absorbable antibiotics in patients undergoing allogeneic HSCT as a method of acute GvHD prophylaxis. Microbiome studies in the modern sequencing era are beginning to challenge the benefit of this practice. In this review, we provide a historical perspective on the practice of GD and highlight findings from the limited number of clinical trials evaluating the use of GD for acute GvHD prevention in allogeneic HSCT patients. In addition, we examine the role of the gut microbiota in allogeneic HSCT in the context of recent studies linking the microflora to regulation of intestinal immune homeostasis. We discuss the implications of these findings for future strategies to reduce acute GvHD risk by selective manipulation of the microbiota.

INTRODUCTION

For many patients with malignant hematologic disorders and bone marrow failure, allogeneic hematopoietic stem cell transplant (HSCT) offers the only opportunity for cure. As advances in donor and recipient selection, conditioning regimens and supportive care measures have led to significant improvements in transplant outcomes, the use of allogeneic HSCT continues to increase as a curative option for non-malignant indications such as primary immunodeficiency syndromes, hemoglobinopathies and congenital metabolic

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CONFLICT OF INTEREST

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disorders. Unfortunately, the development of acute GvHD limits the success of allogeneic HSCT and remains a major cause of treatment failure. Although factors such as the level of HLA matching, recipient age and conditioning regimen greatly influence the incidence, acute GvHD occurs in ~ 40% of transplants even where the donor and recipient are fully matched.[1] Patients with grades III and IV acute GvHD have poor outcomes with ~ 30% and < 5% probabilities of long-term survival, respectively.[2]

Acute GvHD is an inflammatory process thought to be triggered by damage resulting from transplant conditioning regimens. This host tissue damage leads to the release of pro-inflammatory cytokines and activation of host APCs that, in turn, have an enhanced ability to present unshared recipient tissue antigens to the alloreactive donor T cells that mediate acute GvHD.[1] Animal models have demonstrated that the intestinal microflora play a key role in the pathophysiology of acute GvHD as damage to the gastrointestinal mucosa caused by the conditioning regimen leads to the release of bacterial lipopolysaccharides and other 'danger/pathogen-associated molecular patterns' into the systemic circulation that can activate innate immune receptors and cause a cytokine storm.[3] Based on early pre-clinical studies showing that eradication of intestinal bacteria could prevent the development of acute GvHD, many HSCT programs have practiced gut decontamination (GD). This is achieved with the administration of non-absorbable oral antibiotics, and has served as a popular approach to acute GvHD prophylaxis for the past three decades. However, the use of GD is controversial and is not practiced at all stem cell transplant centers. In addition, there is no consensus regarding efficacy or ideal choice of antibiotic regimen. More recent microbiome studies in HSCT patients are beginning to elucidate relationships between the gut microbiome composition and clinical outcomes such as the development of bacteremia, acute GvHD and overall survival.[4,5] The correlation between higher bacterial diversity within the gut microbiome and better clinical outcomes following allogeneic HSCT raises concerns regarding the practice of intestinal decontamination.

HISTORICAL BASIS FOR GD IN ALLOGENEIC HSCT

Studies in the 1970s using murine allogeneic HSCT models demonstrated that resident intestinal bacteria contribute to the pathogenesis of acute GvHD, and that growth suppression or 'eradication' of intestinal bacteria prevented the development of severe acute GvHD even in MHC-mismatched transplants. One of the first well-controlled studies to show that the absence of gut microflora was protective against acute GvHD was performed using germ-free and conventional C3H/He mice transplanted with bone marrow from DBA/2 mice after lethal irradiation.[6] In this MHC-mismatched allogeneic HSCT model, the germ-free allogeneic chimeras had 98% survival at 120 days post irradiation, whereas the conventional allogeneic chimeras had 64% survival at 30 days and 0% survival at 120 days. Although the germ-free allogeneic chimeras did not outwardly exhibit many symptoms of acute GvHD, there was histological evidence of acute GvHD that progressed over time in killed animals. Thus, the authors concluded that the absence of microorganisms within the animal and its environment did not eliminate acute GvHD, but could mitigate its severity. In later experiments, Van Bekkum *et al.*[7] induced acute GvHD by administering bone marrow cells or a mixture of bone marrow cells and spleen cells from C57BL/Rij donor mice into lethally irradiated conventional or germ-free CBA recipient mice. Using this model system,

they similarly demonstrated that conventional mice developed severe GvHD and had significantly decreased 90-day survival compared with germ-free mice (< 5% survival in conventional mice versus 100% survival in germ-free mice). The authors then examined animals in which the microflora had been removed, or replaced with less-pathogenic bacteria. To generate a group of 'decontaminated' mice, conventional mice were fed a mixture of non-absorbable antibiotics including neomycin, streptomycin, bacitracin and pimaricin in their drinking water. GD was confirmed by culturing the bedding and feces on a weekly basis, although the true extent of decontamination cannot be determined by this method owing to limitations in the ability to culture many gut microbes such as the obligate anaerobes. In addition, they introduced 'colonization resistant' flora by feeding into germ-free mice. The colonization-resistant flora contains a mixture of an incompletely identified number of non-pathogenic anaerobic bacteria, and protect against colonization of the intestinal tract by other more pathogenic microorganisms. Both the decontaminated conventional mice and germ-free mice with colonization-resistant flora exhibited almost no symptoms of acute GvHD and had greatly increased 90-day survival compared with conventional mice. To further test the role of the intestinal microflora in mediating acute GvHD, the authors 'conventionalized' germ-free mice by introducing bacteria at early time points after transplantation. Interestingly, these conventionalized germ-free mice did not deteriorate and develop the classic symptoms of acute GvHD. Based on these findings, the authors concluded that the symptoms and mortality of acute GvHD were not due to infectious complications of GvHD-induced tissue damage. Rather, the authors hypothesized that the presence of microflora and bacterial Ags leads to the non-specific stimulation of lymphocytes, which subsequently determines the severity of the GvHD reaction. Later murine studies demonstrated that administration of lipopolysaccharide, present in the outer membrane of enteric gram-negative bacteria, could trigger TNFα production by activated macrophages and exacerbate acute GvHD symptoms.[8,9] These observations formed the basis for the practice of GD for acute GvHD prophylaxis in allogeneic HSCT patients.

EVALUATION OF GD FOR ACUTE GVHD PREVENTION IN ALLOGENEIC HSCT PATIENTS

Few human trials have examined GD with oral non-absorbable antibiotics as a clinical strategy for lowering the risk of developing acute GvHD, and a clear benefit has not been demonstrated (Table 1). The first prospective randomized study to examine the effects of isolation and decontamination procedures in HSCT patients showed that laminar air flow isolation led to significantly less septicemia or major local infections.[10] Patients in the laminar air flow group also received oral non-absorbable antibiotics, sterile diets and skin cleansing. Patients in the 'control' group were placed in single rooms, but otherwise had minimal precautions and did not receive decontamination with oral antibiotics and sterile food. However, patients in this single institution study had poor compliance with the oral antibiotic regimens, and only 9 of 46 patients in the laminar air flow group had complete and sustained suppression of all fecal flora as measured by twice weekly stool cultures with full speciation. In addition, the study population consisted of patients with aplastic anemia and acute leukemia, who received different conditioning regimens and were analyzed separately. Among the patients with aplastic anemia, those randomized to laminar air flow and

decontamination had a longer median time to onset of GvHD compared with patients in the control group ($P = 0.03$). There were no significant differences in the incidence and severity of GvHD. For patients with acute leukemia, no significant differences in GvHD incidence, severity or time to onset were detected. Owing to the small numbers of patients in each subgroup, it was not possible to evaluate the effects of GD on the incidence and severity of acute GvHD. Of the four patients with aplastic anemia who were able to take oral antibiotics and achieve sterile stools, none developed significant GvHD. Five patients with acute leukemia achieved sterile stools, and four developed severe GvHD. A subsequent study from the same institution performed a retrospective analysis on a larger population of 130 patients undergoing allogeneic HSCT with HLA-identical sibling donors for aplastic anemia, of which 39 patients had received laminar air flow isolation and a decontamination regimen consisting of oral non-absorbable antibiotics, sterile food and skin cleansing.[11] Patients in the laminar air flow and decontamination group showed significantly decreased rates of grades II–IV acute GvHD ($P = 0.05$) and increased overall survival ($P = 0.03$). However, as data regarding antibiotic compliance and success of fecal flora suppression were not reported, it is not possible to evaluate the contribution of GD to the reduction in acute GvHD risk. Furthermore, the extrapolation of these findings to the current transplant era is difficult, as sterile diets and skin cleansing are not standard practices in modern HSCT programs.

Several subsequent studies examining correlations between GD and acute GvHD incorporated the collection of stool culture data to measure the efficacy of GD. Vossen *et al.*[12] performed two retrospective studies to examine the association of acute GvHD with the degree of GD. Their first study examined 65 children who underwent matched sibling donor bone marrow transplantation in the Netherlands between 1971 and 1986. Among these patients, a comparison of two different GD regimens (‘complete’ versus ‘selective’) was performed to look for differences in infectious complications and incidence of Grade II or higher acute GvHD. Of note, the patient population was non-uniform between the two comparison groups as there were significantly more patients with acute leukemia in the complete GD group. For either regimen, decontamination was defined as ‘successful’ if the target microorganisms could not be isolated from more than two consecutive stool samples within the GD treatment period (day −7 to +40 after HSCT). Successful decontamination was obtained in a larger proportion of patients in the selective GD group (17 of 21) compared with the complete GD group (12 of 44). Whether or not decontamination was successful, this study reported significantly less acute GvHD (grade II or higher) in the patients who received the complete GD regimen (7/40 in the complete GD group versus 9/18 in the selective GD group, $P < 0.01$). In a more recent retrospective study, Vossen *et al.*[13] examined stool culture results from a more uniform population of pediatric patients transplanted between 1989 and 2002 with the aim of determining whether successful suppression of the intestinal microflora prevented acute GvHD. Decontamination was considered successful when stool cultures were negative for bacterial or fungal species in ≥ 3 of 5 stool samples obtained between days − 10 to +30. Their culture methods were able to identify facultative anaerobic gut microorganisms with a limit of detection of 102 microogranisms/g feces. According to their criteria, 51% (57/112) of the analyzed transplant recipients had ‘successful’ decontamination. Although the authors reported that patients with successful decontamination had a significantly lower rate of acute GvHD, the overall rate of

GvHD was low with only 8% (9/114) of all patients developing acute GvHD. Furthermore, nearly all cases were grade I with only 1 case of grade II acute GvHD.

In a retrospective study of 194 patients undergoing matched sibling allogeneic HSCT between 1975 and 1989, Beelen *et al.*[14] also examined the contribution of intestinal bacterial growth suppression to the risk of grade II–IV acute GvHD. There were differences in isolation conditions and oral antibiotic regimens among the analyzed patients, but all isolation and oral decontamination techniques were supplemented with daily skin sterilization, autoclaved nutrition and sterile beverages. Stool samples were cultured on a weekly basis for aerobic, microaerophilic and anaerobic bacteria, and bacterial growth was quantitated by measurement of colony-forming units. Patients were classified as being 'sustained decontaminated' if continuous bacterial growth suppression was demonstrated in the interval between the day of marrow transplantation and day +35, development of acute GvHD or death, whichever came first. The fraction of patients with 'sustained' decontamination for all bacteria (aerobic and anaerobic) was only 8% (15/194). The authors then looked at the decontamination efficacy, defined as the percentage of germ-free stool samples, by week after transplant and by bacteria type (aerobic, anaerobic and all). Combining all weeks post transplant and all bacteria, the decontamination efficacy was significantly higher in patients with no or grade I acute GvHD compared with patients with grades II–IV acute GvHD ($P < 0.002$). Interestingly, when examined by bacteria type, there was a significant reduction in the incidence of grades II–IV acute GvHD in patients with sustained decontamination of anaerobic bacteria ($P < 0.006$). There was no significant association between sustained decontamination of aerobic bacteria and acute GvHD incidence. In a multivariate analysis of risk factors for acute GvHD, the lack of sustained anaerobic decontamination remained a significant independent prognostic factor for grades II–IV acute GvHD (RR 1.7, 95% CI 1.2–2.5, $P < 0.002$). However, only 21% of patients in this analysis achieved a sustained decontaminated state with regard to anaerobic bacteria. Consequently, Beelen *et al.*[15] conducted a prospective randomized trial comparing GD using ciprofloxacin and metronidazole, for improved anaerobic coverage, versus ciprofloxacin alone as a GD regimen. As expected, the metronidazole-containing arm had a significantly higher proportion of stool samples with no detectable anaerobic bacteria ($P < 0.00001$). Similar to their previous study, patients who developed grades II–IV acute GvHD had less successful decontamination of anaerobic bacteria in their stool samples than patients who had no or grade I acute GvHD ($P < 0.005$). The cumulative probability of grade II–IV acute GvHD was significantly lower in the metronidazole-containing arm (25% versus 52% in the ciprofloxacin alone arm, $P < 0.002$). However, when the analysis was broken down by donor type, the influence of metronidazole on acute GvHD incidence was significant only in recipients of transplants from HLA-identical sibling donors. Nevertheless, the findings from this single-center trial imply that treatment with metronidazole and the potential preferential eradication of 'culturable' anaerobic bacteria can reduce the risk of acute GvHD. These observations are supported by a recent microbiome study that found an enrichment of enterococci in post-transplant stool specimens from allogeneic HSCT patients who developed gut GvHD compared with patients without gut GvHD.[16]

To date, the clinical trials evaluating the benefit of GD for acute GvHD prophylaxis are limited to single-center studies, and often do not have a comparison group that did not

receive any GD. These trials are also complicated by the lack of a uniform GD regimen, poor compliance and varying measures of successful decontamination. Moreover, the standard culture methods, used in the era before next-generation sequencing technologies, were limited in their ability to detect many difficult-to-culture microorganisms. Thus, stool samples previously described as ‘sustained decontaminated’ or ‘germ-free’ by culture methods may lead to different results if analyzed by today’s culture-independent methods. Many centers also use GD to decrease the risk of bacteremia in the setting of neutropenia and mucositis in HSCT patients. Similarly, it is difficult to assess the efficacy of GD in bacteremia prophylaxis because of widely varying antibiotic regimens between centers and the lack of randomized controlled trials comparing GD to placebo.[17] Owing to insufficient evidence, GD is not routinely recommended for the prevention of acute GvHD or bacteremia.[18] In an informal survey of adult and pediatric transplant centers in the United States, only 3 of 8 adult centers and 4 of 10 pediatric centers routinely practiced GD for allogeneic transplant patients (Table 2). Moreover, the approaches used for GD in these centers varied widely. With limited and inconsistent clinical evidence, the use of GD for acute GvHD prophylaxis in patients undergoing allogeneic HSCT remains controversial, and there is no consensus regarding efficacy or ideal choice of antibiotic regimen. In the following section, we will review recent microbiome studies that provide novel insights into the role of the intestinal microflora in immune homeostasis that may have important implications for the use of GD in HSCT patients.

THE ROLE OF INTESTINAL MICROBIOTA IN MAINTAINING IMMUNE HOMEOSTASIS

Although the early studies in murine models of allogeneic HSCT described above indicated that eradication of the gut microbiota was protective against inflammation and acute GvHD, more recent studies have demonstrated that commensal bacteria are critical to maintaining immune homeostasis in the intestine. Much of our understanding about the relationship between commensal gut flora and the mucosal immune system comes from studies in murine models of inflammatory bowel disease. Similar to the early studies in murine models of acute GvHD, it was observed that germ-free rodents did not develop colitis,[19] antibiotic therapy could prevent and treat colitis symptoms[20] and introduction of either aerobic and anaerobic non-pathogenic commensal bacteria into germ-free mice induced colitis.[21] More direct interactions between the gut microflora and CD4+ T cells were demonstrated in a transfer colitis experiment using the C3H/HeJBir strain of mice, which spontaneously develop colitis.[22] This study first showed that CD4+ T cells isolated from spleen and mesenteric lymph nodes of C3H/HeJBir mice did not respond to stimulation by food or epithelial cell antigens, but had a robust proliferative response to stimulation by antigen-presenting cells pulsed with lysates of cecal bacteria. When transferred into *scid* mice, the CD4+ T cells activated by bacterial antigens led to the development of colitis, whereas CD4+ T cells activated *in vitro* with monoclonal anti-CD3 did not result in colitis. Subsequent studies have suggested that distinct components of the gut microbiota may induce specific lymphocyte subsets. For example, segmented filamentous bacteria have been found to influence differentiation of IL-17-producing T-helper cells, which have

inflammatory potential and mediate host resistance against intestinal pathogens as well as systemic autoimmunity.[23,24]

Using a similar adoptive transfer colitis model, Strauch *et al*.[25] have suggested that intestinal bacteria are also involved in the induction of anti-inflammatory, regulatory T cells (Treg). The authors used a previously established model in which the transfer of predominantly naive T lymphocytes into T- and B-cell-deficient *scid* mice induces severe colitis. The transferred T cells proliferate within the lymphopenic host and lead to inflammatory Th1 effector responses, which can be prevented by the co-transfer of mature T cells thought to contain Treg subpopulations.[26] First, to investigate whether the gut flora of the donor mice is necessary to prime T cells to mediate intestinal inflammation within this transfer model of colitis, T lymphocytes were isolated from donor mice housed under germ-free or conventional conditions. The transfer of predominantly naive (CD4+ CD62L+) T lymphocytes from germ-free mice into *scid* mice led to the rapid development of severe colitis, whereas *scid* mice reconstituted with T cells isolated from conventionally housed animals were initially healthy and slowly developed clinical signs of colitis. In addition, the co-transfer of mature (CD4+ 62L −) T cells isolated from conventional mice, but not from germ-free mice, were able to mitigate the colitis producing potential of the naive (CD4+ CD62+) T cells. The authors hypothesized that an increased proportion of anti-inflammatory Tregs may underlie the functional differences observed in the T lymphocytes isolated from the conventionally housed mice. Further phenotypic analysis of the CD4+ CD62L+ T cells indicated higher expression of the glucocorticoid induced TNF receptor-related protein (GITR), a marker for Tregs, in the conventionally-housed mice. Analysis of the CD4+ CD62L − T-cell subsets showed that the CD4+ T cells isolated from the conventional mice secreted higher levels of the anti-inflammatory cytokine, interleukin-10, and expressed higher levels of FoxP3, a transcription factor associated with the development of Tregs. Indeed, following up on these observations, several groups in the past 5 years have identified bacterial species and bacterial metabolites that induce the differentiation and expansion of extrathymic Tregs. For example, monocolonization of germ-free mice with the Gram-negative anaerobe and human symbiont *Bacteroides fragilis* leads to a significant increase in the percentage of interleukin-10-producing FoxP3+ Tregs within the colonic lamina propria.[27] Polysaccharide A, a surface molecule produced by *B. fragilis* with immunomodulatory properties, is sufficient to expand functional FoxP3+ Tregs systemically and to protect and cure animals from experimentally-induced colitis.[28,29] By examining Treg induction in antibiotic-treated specific pathogen-free mice and germ-free mice inoculated with chloroform-resistant fecal microorganisms, another group identified Clostridia (Gram-positive and spore-forming bacteria) as an important component of the intestinal flora with the ability to induce interleukin-10-producing colonic Tregs.[30] The same group then showed that oral administration of a mixture of 17 Clostridia strains with high Treg-inducing potential was able to alleviate colitis and diarrhea symptoms in experimental colitis models.[31] Several independent analyses of bacterial metabolites present in the feces of germ-free versus specific pathogen-free mice further narrowed down the Treg-inducing activity to short-chain fatty acids.[32–34] More recent studies continue to uncover layers of complexity involved in the regulation of immune homeostasis by showing that microbiota-induced Tregs express the nuclear hormone receptor retinoic acid receptor-related orphan

receptor γt (RORγt), which was previously thought to promote differentiation of pro-inflammatory T-helper cells.[35] These RORγt+ Tregs appear to control Th2, T-helper and Th1 responses, and differ in gene expression profile and function from GATA3+ Tregs, which do not respond to short-chain fatty acids. Rather, GATA3+ Tregs express ST2 and respond to IL-33/alarmin, a cytokine that is released in response to tissue damage.[37] Thus, gut microbes appear to regulate intestinal homeostasis by influencing the balance between different Treg subtypes (Figure 1).

The practice of GD for acute GvHD prophylaxis in HSCT patients should be considered carefully in the setting of these observations. A better understanding of the molecular pathways by which commensal bacteria in the intestine modulate the balance between pro- and anti-inflammatory T cells may lead to strategies for more selective manipulation of the gut microbiome in HSCT patients, and may identify new drug targets for the reduction of inflammation via enhancement of intestinal Tregs. Evidence from murine studies suggests that metabolites generated by the gut microbiota not only regulate intestinal immune homeostasis, but also influence circulating immune cells.[33] In addition, the majority of Tregs in the colon are derived from thymus-generated Tregs.[38] Experiments using transgenic mice expressing a photoconvertible fluorescent reporter demonstrated that all of the major myeloid and lymphoid immune cell subsets can migrate between the gut and distal lymph nodes.[39] Together, these findings have important implications for the role of the gut microbiome during immune reconstitution in the post-HSCT setting. At present, it is not known how the practice of GD impacts composition of the gut microbiome following HSCT and how this in turn affects recovery of the immune system.

GUT MICROBIOME STUDIES IN HSCT PATIENTS AND CORRELATIONS WITH CLINICAL OUTCOMES IN THE MODERN ERA

Allogeneic HSCT provides a unique setting in which to study the gut microbiome and its interactions with the mucosal and peripheral immune system. Gastrointestinal GvHD is primarily an allo-immune response as opposed to a general inflammatory response. For example, patients undergoing autologous HSCT are also exposed to high-dose chemotherapy as part of their conditioning regimen, which leads to mucosal damage and inflammation. These patients often develop diarrhea, but do not develop the pathologic features of gastrointestinal GvHD. In addition, many patients undergoing allogeneic HSCT do not develop gastrointestinal GvHD, indicating that despite the presence of alloreactive T cells, it is possible to suppress their function and induce immune tolerance in the gut. Based on the studies described in the previous section, the gut microbiota may have an important role in this process. However, previous studies of GD and its impact on the intestinal microflora in HSCT patients were limited in their ability to characterize the microbiome. In these studies, confirmation of ‘successful’ or ‘sustained’ decontamination was based on qualitative stool culture methods. For example, the aerobic culture method used by Beelen *et al.* allowed monitoring of *Escherichia coli*, klebsiella, proteus, enterobacter, pseudomonas, enterococcus, lactobacillus and staphylococcus species. Their anaerobic culture methods were able to detect species of the genera bifidobacterium, bacteroides, fusobacterium, clostridium, eubacterium, peptococcus, peptostreptococcus and veillonella.[14] Although

laboratory culture methods are able to identify an extensive panel of bacterial and fungal species, there remain a large number of species that are difficult or impossible to culture under laboratory conditions. In addition, stool culture methods allow for identification of the various species contained within the sample, but cannot provide information regarding relative abundance within the total population. With the advent of modern sequencing technologies, microbiome characterization no longer relies on laboratory culture techniques and can provide comprehensive and quantitative information about all species that are present.

The most common tool used for taxonomic characterization of a population of microbes is 16S ribosomal DNA sequencing.[40] The 16S ribosomal DNA gene has both highly conserved and variable regions. By amplifying regions of the gene using primers against the conversed regions, one can generate a population of bar-coded sequences that contain the region internal to those two primers, which is highly variable. These variable regions can then be sequenced and classified taxonomically based on similarity to a reference database. This method, though powerful, is limited by the fact that prokaryotes contain a differing copy number of 16S genes (over about an order of magnitude) and many 16S sequences have yet to be associated with a known taxon.[41] With advances in sequencing technology and throughput, efforts to characterize populations of organisms taxonomically have started to take advantage of shotgun sequencing approaches.[42,43] It is now possible to characterize the entire ‘metagenome’, or DNA sequences of all of the microorganisms within a particular human niche. Yet, only a subset (our preliminary data suggests 20–40%) of the organisms present within the human gut microbiota have been ‘whole genome sequenced’, or have defined 16S ribosomal DNA sequences that would allow taxonomic classification.

Recent studies describing the composition of the gut microbiome and correlations with clinical outcomes are beginning to provide entry points for studying the role of the microbiome in the establishment of immune tolerance in the setting of allogeneic HSCT. In 2014, Taur *et al.*[44] reported that low gut microbiota diversity, as measured by 16S ribosomal DNA sequencing, is clearly associated with increased mortality in HSCT patients. Additional studies provided greater support for the concept that certain microbiota compositions may be associated with clinical outcomes. For example, Jenq *et al.*[5] demonstrated that the presence of bacteria from the genus blautia in post-HSCT patients was associated with decreased GvHD-associated mortality. In another report, post-HSCT patients with gut microbiota dominated by enterococcus or proteobacteria had a nine- or fivefold increase in bacteremia with vancomycin-resistant enterococcus or Gram-negative bacteria, respectively.[4] It will be interesting to examine whether microbiota composition has any associations with relapse in patients undergoing HSCT for hematologic malignancies. Recent murine studies have shown that tumors in antibiotic-treated or germ-free mice can be more refractory to chemotherapy due to decreased cytokine production by tumor-infiltrating myeloid cells or due to decreased T_H17 cell induction by specific Gram-positive bacteria.[45–47] Based on these observations, it is possible that microbiota composition could modulate the immune-mediated graft-versus-leukemia effect.

CONCLUSIONS AND FUTURE DIRECTIONS

The field of microbiota research is undeniably booming, with many researchers seeking to characterize the diversity within microbial niches such as human skin, oral cavity and stool. Although the detailed cataloging of species is fundamental to microbiota research, there is a growing interest in understanding how these organisms interact with the human host to induce or facilitate states of health and disease. Although positive and negative associations between the microbiota composition and host health have been widely reported, the mechanisms whereby the microbiota affects host health are incompletely understood.[48–56] To begin addressing more mechanistic questions, many in the field have devised methods to quantify the abundance of various biochemical pathways in microorganisms by counting the number and types of genes that are predicted to be encoded by the DNA that is sequenced. Although this method may provide a good approximation, it makes the assumption that all sequences that are present are actively transcribed and result in functional changes in the biology of the organism. Others have developed metatranscriptomic methods—the large-scale sequencing of mRNAs extracted from microbial communities.[57,58] Although they have the potential to reveal interesting insights into microbial activities and their regulation, these methods are challenged by the fact that most prokaryotic mRNAs are not extensively polyadenylated, and are thus quite unstable, degrading quickly after RNA extraction.

Next-generation sequencing technologies have revolutionized the microbiome field by enabling culture-independent approaches to characterizing microbiota composition and diversity at a high resolution. Consequently, enormous data sets have been generated describing the microbiome composition in multiple body sites across many individuals and in various disease states.[59] Analysis of these data is leading to important discoveries in the biology of host-microbe interactions and how these relationships impact health and disease states. The application of modern microbiome studies within the HSCT field has revealed correlations between the gut microbiome composition and clinical outcomes such as overall survival and GvHD-related mortality. The common practice of GD for acute GvHD and bacterial prophylaxis in HSCT patients should be re-examined In light of these recent findings. Previous studies of GD in HSCT patients have been limited by small sample sizes with heterogeneity of patient characteristics, non-uniformity of GD regimens and reliance on stool culture methods to determine the extent of decontamination. It will be important to evaluate the clinical benefit of GD in HSCT patients using randomized controlled clinical trials with homogenous patient populations and with the application of comprehensive molecular approaches to the characterization of the gut microbiome. It will also be informative to understand the contribution of nutrition, specifically exposure to complex diets replete with microbially accessible carbohydrates such as fiber, to maintenance of gut microbiome diversity during HSCT. Continued characterization of the gut microbiome composition throughout the peri- and post-transplant period will be important for identifying microbiome ‘signatures’ that are associated with clinical outcomes such as relapse, acute and chronic GvHD and overall survival. In conjunction with the identification of favorable microbiome signatures, understanding how different conditioning or GvHD prophylaxis regimens impact the precise composition of the intestinal microbiome and its recovery will help to inform individualized treatment programs. Given the recent findings linking gut

microbiota to regulation of immune homeostasis, it will also be important to study how recovery of the gut microbiome influences reconstitution of the immune system post transplant. Fulfillment of these aims will lead to the development of therapeutic interventions such as tailored antibiotic regimens, dietary modifications or supplements, or bacterial supplements that can modify the gut microbiome and favorably influence immune reconstitution to optimize outcomes following HSCT.

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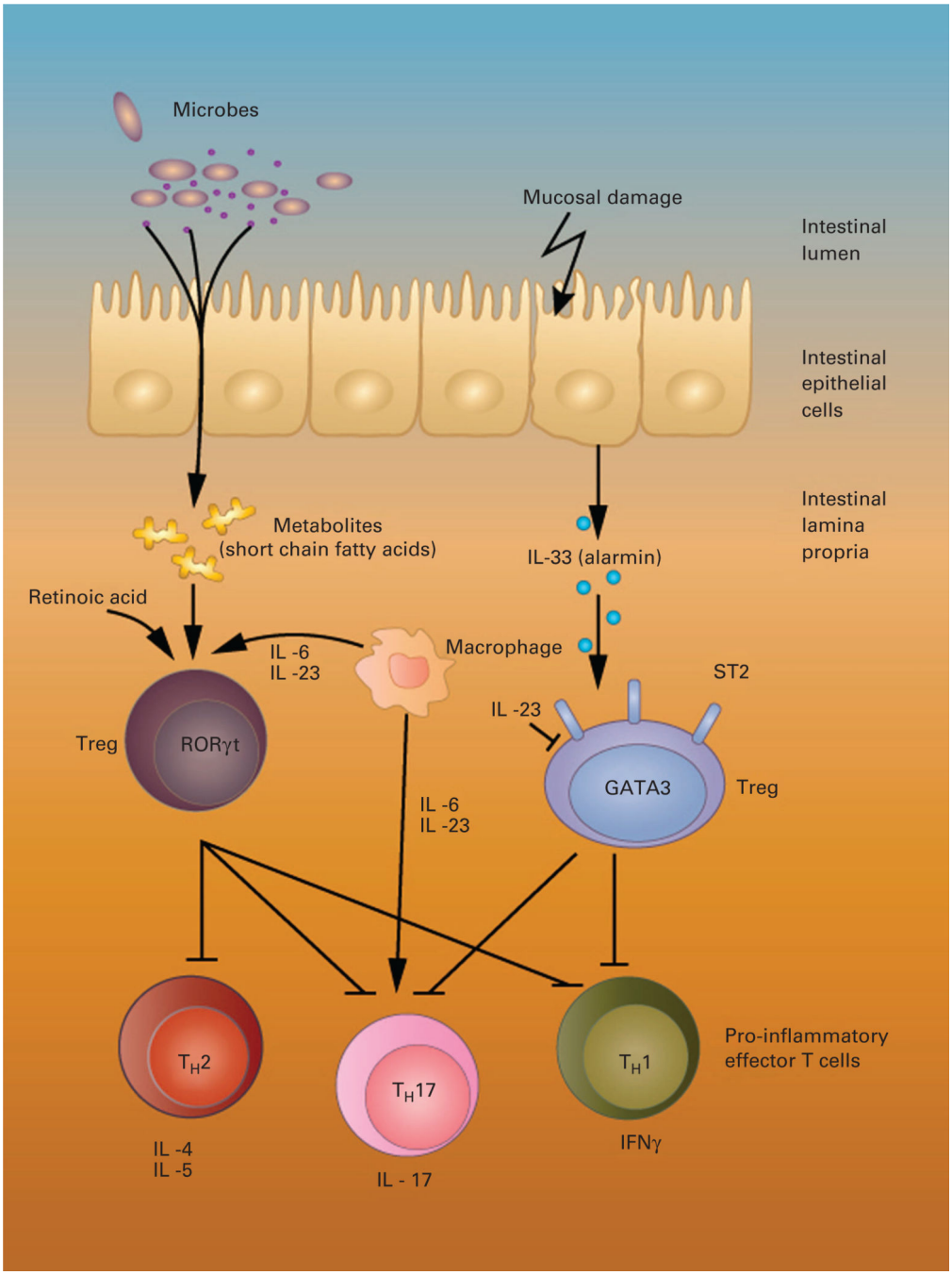

Figure 1.

Complex regulation of T-cell homeostasis in the intestine. (Adapted from Hegazy and Powrie, *Science* 2015).[60] Signals from the gut microbiota and tissue damage regulate a balance between RORγt and GATA3-expressing Tregs in the mouse intestine. RORγt+ Tregs are induced by microbe-derived signals such as short-chain fatty acids and retinoic acid, and also expand in response to cytokines (IL-6 and IL-23) previously known to induce Th17 cells. The RORγt+ Tregs have been implied in the suppression of Th2, Th17 and Th1 responses. GATA3+Tregs are not microbe-responsive and express the IL-33 receptor, ST2.

Mucosal tissue damage leads to the release of IL-33, which in turn induces GATA3+ Tregs to coordinate healing by blocking Th1 and Th17 responses. Whereas IL-23 induces RORγt+ Tregs, this cytokine inhibits ST2 signal transduction and blocks GATA3+ Treg responsiveness to IL-33.

Table 1

Summary of gut decontamination trials in alloHSCT patients

Year (ref.)	Type of study	Patient population (n)	Comparison groups	GD regimen (all oral antibiotics)	Impact on acute GvHD incidence
1978[9]	Prospective, randomized; single-center	Adults undergoing alloHSCT with HLA-identical sibling donors for aplastic anemia or acute leukemia (90)	LAF isolation with decontamination vs control (single room, minimal precautions, no decontamination)	Variable, but included: gentamicin, mycostatin, vancomycin, paromomycin, polymyxin	No difference in incidence, but significant difference in median time to onset of GvHD (48 days in LAF vs 24 days in control, $P = 0.03$) in aplastic anemia patients. No difference in incidence or time to onset of GvHD in acute leukemia patients.
1983[10]	Retrospective analysis	Adults undergoing alloHSCT with HLA-identical sibling donors for aplastic anemia (130)	LAF isolation with decontamination vs control (single room, minimal precautions, no decontamination)	Not described	Significantly lower cumulative grade II–IV GvHD incidence ($P = 0.05$) and significantly higher probability of survival ($P = 0.03$) in LAF with decontamination group.
1990[11]	Retrospective analysis	Pediatric patients undergoing alloHSCT with HLA-identical sibling donors for bone marrow failure or leukemia (65)	Two different GD strategies (‘complete’ vs ‘selective’)	‘Complete’ GD: neomycin, polymyxin B, cephalodorin, amphotericin B; ‘Selective GD’: nalidixic acid, co-trimoxazole, polymyxin B, neomycin, amphotericin B	Significantly lower incidence of ≥ grade II acute GvHD in ‘complete’ GD group ($P < 0.01$).
1992[13]	Retrospective analysis	Mostly adult patients undergoing alloHSCT with HLA-identical sibling donors for aplastic anemia or leukemia (194)	‘Sustained’ vs ‘Not sustained’ GD based on bacterial growth from stool cultures	Gentamicin/tobramycin or netilmycin in combination with amphotericin B and nystatin. First 22 patients also received oral cephazolin solution.	Significantly lower incidence of grade II–IV acute GvHD in patients with ‘sustained’ decontamination of anaerobic bacteria ($P < 0.006$).
1999[14]	Prospective, randomized; single-center	Mostly adult patients undergoing alloHSCT with HLA-identical sibling, mismatched family and matched unrelated donors for hematologic malignancies (134)	Two different GD strategies (institutional standard vs additional anaerobic coverage)	PO ciprofloxicin alone vs PO ciprofloxacin and PO metronidazole	Significantly lower incidence of Grade II–IV acute GvHD in the metronidazole-containing arm ($P < 0.0005$) among recipients of transplants from HLA-matched sibling donors. No difference between the two treatment arms in recipients of mismatched family or matched unrelated donor transplants.
2014[12]	Retrospective analysis	Pediatric patients undergoing alloHSCT with HLA-identical sibling donors for leukemia (112)	‘Successful’ vs ‘unsuccessful’ GD based on bacterial growth from stool cultures	Amphotericin B and gentamicin, plus cefaloridin (1988–1993) or IV ceftriaxone and PO vancomycin (1993–1995) or PO piperacillin/tazobactam (after 1995)	Acute GvHD in 1 of 57 patients with ‘successful’ decontamination (grade I) and 8 of 55 patients with ‘unsuccessful’ decontamination (1 grade II, 7 grade I), $P = 0.013$.

Abbreviations: alloHSCT = allogeneic hematopoietic stem cell transplantation; GD = gut decontamination; LAF = laminar air flow; PO = per os.

Table 2

Informal survey of GD practices at U.S. centers

Center	Gut decontamination regimen
Adult	
Center 1	PO polymyxin B and nystatin
Center 2	PO ciprofloxacin
Center 3	PO levofloxacin
Centers 4–8	No gut decontamination
Pediatric	
Center 1	PO vancomycin and polymyxin B
Center 2	IV metronidazole
Centers 3–4	PO levofloxacin
Centers 5–10	No gut decontamination

Abbreviations: IV = intravenous; PO = per os.