

79091\_Auto\_Edited.docx

**Name of Journal:** *World Journal of Hematology*

**Manuscript NO:** 79091

**Manuscript Type:** MINIREVIEWS

**Advantageous tactics with certain probiotics for the treatment of graft-versus-host-disease after hematopoietic stem cell transplantation**

Sayuri Yoshikawa, Kurumi Taniguchi, Haruka Sawamura, Yuka Ikeda, Ai Tsuji, Satoru Matsuda

**Abstract**

Hematopoietic stem cell transplantation (HSCT) becomes a standard form of cellular therapy for patients suffering from malignant diseases. HSCT is the first-choice of immunotherapy, although HSCT can be associated with many complications such as graft-versus-host disease (GVHD) that is a major cause of morbidity and mortality after allogeneic HSCT. It has been shown that certain gut microbiota could be applied for protective and/or regenerative immunomodulatory effects by the production of short-chain fatty acids (SCFAs) such as butyrate in the experimental models of GVHD after allogeneic HSCT. Loss of gut commensal bacteria which can produce SCFAs may worsen dysbiosis, which could increase the risk of GVHD. Expression of G-protein coupled receptors such as GPR41 seems to be upregulated in the presence of commensal bacteria, which might be associated with the biology of regulatory T cells (Tregs). Treg cells are a suppressive subset of CD4 positive T lymphocytes implicated in the prevention of GVHD after allogeneic HSCT. Here, we would like to discuss the current findings of the relationship between the modification of gut microbiota and the GVHD related immunity, suggesting that tactics with certain probiotics for the beneficial symbiosis in gut-immune axis might lead to the elevation of safety in the allogeneic HSCT.

## INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a broadly accomplished curative therapy for several hematological diseases, which is achieved by circulatory infusion of hematopoietic stem cells to the patients from human leukocyte antigen (HLA)-matched allogeneic donor or from the autologous patient themselves [1]. **(Figure 1)** However, **the HSCT techniques are actually restricted** by potentially life-threatening complications, and one of the most serious complications is graft *vs* host disease (GVHD) [2], **which is a pathogenic condition that arises when immune cells of the graft might systematically distinguish the host as foreign enemy, and affect the recipient's tissues/organs** in the body induced by the influx of donor-derived effector T cells into peripheral tissues [3].

**(Figure 1)** **3** The pathophysiology of GVHD may include donor T cells and/or inflammatory cytokine-mediated injury to patient's tissues as a result of transplant and/or conditioning regimen. Immune reactions underlying **the GVHD may also include greater proliferation and/or migration of active immune cells to the target tissue or organ**. Several organs could be targeted by the GVHD. Therefore, the patient should be prepared with rigorous chemotherapy and/or radiotherapy to reduce immunological resistance in addition to extinguish residual malignant cells before HSCT.

Remarkably, several risk factors in gut might play important roles for the initiation of GVHD [4]. Gut microbiota has been hypothesized to have a role in GVHD onset [5]. In addition, the path of gut microbiota to the recovery following HSCT might be related to the risk of developing a GVHD [6]. It has been suggested that potential modifiable targets of gut microbiota could reduce the risk of GVHD [4, 7]. For example, a prolonged gut microbiota-dysbiosis following HSCT has in turn been demonstrated to an increased risk of innate immune system activation and/or systemic infections, causing the development of GVHD [8]. Equally, the prompt recovery of gut microbiota may provide the host protective against the onset of GVHD by the keen preservation of immune homeostasis [9]. The gut flora can make the difference when it comes to allogenic HSCT [10]. **4** As prevention and/or treatment of the GVHD are the imperative issue for improving the

efficacy of HSCT, it is significant that homeostasis of gut microbiota could possibly reduce the risk of GVHD.

## 2. Reprogramming of immune cells and GVHD

GVHD is generally characterized by cytokine production, proliferation, and migration of reactive T cells of donor. Therefore, oxidative stress is frequently elevated in the tissues/organs of recipients with allogeneic HSCT, which may further contribute to the progression of the GVHD [11]. Patients with allogeneic HSCT may have various risk factors for developing a GVHD such as acute kidney injury [12]. In particular, GVHD is a chief risk factor for the development of renal failure and/or acute kidney injury in HSCT recipients [13]. The other risk factors are sepsis, nephrotoxic medications including amphotericin B and/or cyclosporine [13]. A reprogramming of immune cells might be a feature of GVHD, which is connected with the differentiation of CD4+ cells to the pathogenic type 1 T helper (Th1) and type 17 T helper (Th17) cells as well as the insufficiency of the immune-suppressive regulatory T cells (Tregs) [14]. (Figure 1) In addition, the reprogramming of cellular metabolism is also a feature of GVHD, showing that mTOR inhibition may reduce the GVHD and increases the potency of peripheral Tregs as well as induction of Tregs from CD4 positive T cells [14, 15]. The immuno-metabolic effects might be purposed for metabolic management of GVHD. In fact, it has been shown that the metabolic reprogramming might represent a promising strategy for the therapeutic target of GVHD [16, 17]. Following the HSCT, donor T cells are stimulated by the antigens of mismatched recipient cells to undergo glycolytic metabolic reprogramming and form allogeneic effector T cells [18].

Metabolic cellular regulation is important for immune-regulation, and several cytokine production and/or metabolic condition of HSCT recipients have been revealed as a risk of GVHD development [19]. Previous studies have suggested that differences in metabolism of immune cells have significant association with the pathology of GVHD [20]. T cells could undergo distinct metabolic reprogramming in response to allogeneic antigens, suggesting that the reactive T cells might depend on glycolysis to meet ATP

demands [21]. It is indispensable to evaluate the metabolic reprogramming of T cells in order to appropriately respond to the allogenic antigens after HSCT [21]. On the other hand, it has been shown that Tregs could suppress the reactive T cells responsible for the GVHD and/or allogenic graft rejection [22]. Therefore, the immune-suppressive donor Tregs could considerably prevent a GVHD and/or a graft rejection [23]. In fact, adoptive transfer of human Tregs has been revealed significant efficacy in preventing GVHD after allogeneic HSCT [24].

Blockade of the nutrient sensor mechanistic target of rapamycin (mTOR) through the use of its antagonist, such as rapamycin, is additional key aspect of metabolic reprogramming in GVHD [25]. Mechanistic target of rapamycin complex 1 (mTORC1) is sensitive to rapamycin inhibition, while mTORC2 is not [25]. Hyper-activation of mTORC1-signaling might be necessary for the pathogenesis of inflammatory bowel disease which is multi-factorial chronic intestinal inflammation driven by pathogenic T cells [26]. Tregs are tightly controlled by the activation of nutrient-fueled mTORC1 [27]. Inhibition of mTORC1 may protect the bioactivity and/or homeostasis of human Tregs from apoptosis [28]. Cytokine situation towards Th17 over Treg immunity could be found through impaired autophagy by decreased mTORC1 [29]. It has been shown that the mTORC1 could drive the proinflammatory expansion of Th1, Th17, and double-negative T cells, and might inhibit the development of Tregs [30]. (Figure 2)

### 3. Promising roles of gut-microbiota for the treatment of GVHD

Gut microbiota might be associated with the development of GVHD, in which loss of diversity in the microbiota could be a risk factor for the GVHD [31, 32]. Gut microbiota might be perturbed due to basic HSCT conditioning, various infections, and/or use of antibiotics, resulting in increased inflammatory factors influencing the Treg/Th17 balance, thus promoting the development of GVHD. It is noteworthy that these inflammatory factors are associated with the Treg/Th17 balance. Therefore, gut microbiota with inflammatory diseases could affect GVHD and/or the balance of Treg/Th17 [33, 34]. In general, diet has an influence on the construction of gut microbiota.



In particular, the activities of gut microbiota may rely on a well-adjusted<sup>12</sup> production of short-chain fatty acids (SCFAs) such as acetate, propionate and/or butyrate, which are mainly the products of gut fermentation of non-digestible polysaccharides such as cellulose and/or resistant starch in vegetables<sup>6</sup> [35]. SCFAs have been recognized as mediators of immune responses, including pathways of cytokine production, which is important to alleviate the risk of GVHD [36]. It has been shown that SCFAs are effective antimicrobial and/or anti-inflammatory compounds supporting the epithelial barrier for metabolic homeostasis in the host [37]. Therefore, some microbial metabolites including the SCFAs might work as protections against the GVHD by adjusting immune-reactions [38]. Remarkably, increased production of microbiota-derived SCFAs could improve the Treg/Th17 balance [39]. In addition, elevated production of SCFAs may lead to the enhanced Treg generation and the suppressed Th17 development<sup>6</sup> [40]. Moreover, SCFAs may up-regulate the production of anti-inflammatory cytokines resulting in the induction of Tregs [41]. Therefore, loss of beneficial gut commensals that can produce SCFAs might affect the severity of GVHD. Clinical scale production of human Tregs might be actually complex and difficult in another way. It has been shown that certain microbiota strains with the high production of butyrate could decrease GVHD [36, 42]. Interestingly, the butyrate, isovaleric acid, and/or branched-chain fatty acids (BCFA) could activate mTORC1 in hepatocytes, suggesting a diet could potentiate the mTORC1 *via* the alterations in gut microbiota [43]. In addition, it has been indicated that GPR41, a G protein-coupled receptor for SCFAs including butyrate, could evoke the mTORC1 phosphorylation [44]. On the contrary, the specific modification of microbial metabolites could have another effects on the condition of GVHD, suggesting that an unrecognized role of microbial metabolites has beneficial effects on GVHD [36].

<sup>3</sup> Gut microbiota possess the great capability to produce D-amino acids which are applied as nutrients to keep bacterial growth and to control spore sprouting<sup>3</sup> [45]. In general, various bacterial species could produce racemases that convert L-amino acids to D-amino acids [46]. Accordingly, higher D-amino acids levels have actually been<sup>8</sup> linked to the mass of gut microbiota [47]. Interestingly, D-serine suppressed the

proliferation of activated CD4 positive T cells and limited their ability to differentiate to Th1 and/or Th17 cells [48]. Consequently, D-amino acids could be effective in preventing GVHD [49]. Similarly, it is well known that disruption of gut microbiota-diversity could exacerbate GVHD [50]. Probiotics and/or prebiotics could also activate the growth of several microorganisms in the gut for health profits of the host [51]. Remarkably, a low diversity of microorganisms might diminish the favorable effect of prebiotics [52]. Therefore, elucidation of the structures, functions, and/or activities of gut microbiota in the host might contribute to the safety of various treatment in HSCT. Undoubtedly, intervention tactics including prebiotics, probiotics, and/or fecal microbiota transplantation (FMT), directing the gut microbiota could become potential new treatment options for the GVHD. (Figure 3)

#### 4. Probiotics as a nutritional support for beneficial gut-immune axis

Gut microbiota consists of a numerous multispecies community that may establish symbiosis with the host, which are defined as constructively functional microorganisms with a health benefit on the host [53]. The gut microbiota are impacted by many factors such as diet, use of antibiotics, and/or geographic environment [54]. Changes in the microbiota are known to be affected by those dietary and/or environmental factors, which have been revealed to initiate redox signaling within the gut mucosa cells [55]. Mechanisms of redox signaling might lead to the result of an inflammatory incident. Thus, the gut microbiota may be also arrested in a sophisticated balance at this viewpoint. Consequently, gut microbiota, ROS, oxidative stress, inflammation, and several inflammatory diseases might be closely associated with each other. In fact, perturbations in the microbial balance may be associated with the initiation of inflammatory bowel diseases [56]. The ROS are defined as oxygen hugging energetic molecules capable of reacting with various organic molecules in a cell, which are also derived from inflammatory reactions [57]. Generally, living cells reluctantly release ROS for essential ATP synthesis, which may cause DNA damage in cells [58, 59]. Some important physiological roles of ROS include the regulation of enzymes involved in autophagy,

DNA synthesis, and/or DNA repair [60]. Certain degrees of ROS could change the signaling pathways to control mRNA and/or protein expression, which governs the cell destiny either cell survival or death [58, 61]. Therefore, gut microbiota might also govern the cell destiny in some cases [62]. Understanding redox regulation of physiological processes seems to be important for developing therapeutic approach with gut microbiota. In addition, patients who undergo HSCT are often suffering from nutritional deficiencies with weight loss possibly due to treatment side effects [63]. Accordingly, nutritional support after the HSCT for the beneficial gut microbiota has become a strategic aspect to be considered.

Probiotics, a procedure for the manipulation of gut microbiota, are active microorganisms that have been deliberated to nutritionally contribute to the host health with adequate amounts of administration. Most probiotics are firstly isolated from healthy human individuals and estimated to be safe. It has been revealed that probiotics could reduce inflammation and/or oxidative stresses [64]. For example, probiotics with certain bacteria such as *Akkermansia* and *Lactobacillus* could alleviate systemic metabolism in inflammatory diseases [65]. Therefore, probiotics with administration of *Lactobacillus plantarum* is reasonable to minimize the risk of developing a GVHD in patients undergoing HSCT [66]. Probiotics could inspire the gut immune system. For example, the immune-stimulatory effects of several *Lactobacillus* species have been discovered. In addition, *Lactobacillus gasseri* and *Lactobacillus johnsonii* could alter the enteric cytokine production by activating dendritic cells [67]. Probiotics could be utilized in treating various diseases. Gut microbiota could induce the maturation of dendritic cells and/or the differentiation of naive T cells into several lymphocyte subsets including Th17 and/or Treg cells [68]. Th17 cells are particularly affected by the abundance of specific commensal bacteria [69]. In the homeostasis of gut microbiota, non-pathogenic bacteria may play a significant role in the stability of adaptive immunity by the regulation of Treg cells [70]. Diet-induced shifts in microbiota composition might have insightful effects on the host immunity especially on T cells [71]. Curiously, high salt intake with diet could drive autoimmunity by inducing Th17 cells [72]. As mentioned above, production of D-amino



acids might be correlated with a relative profusion of bacterial species with specific racemases in the gut microbiota [73]. Higher levels of D-amino acids with the higher mass of gut microbiota may be suggesting that increased abundance of such bacteria is associated with a stressed gut environment for the recovery [47, 74]. In addition, different bacterial species may produce distinct profiles of D-amino acids [75]. Successful alteration in the composition of gut microbiota might be considered as an innovative therapeutic tactics [76]. D-amino acids and ROS- biosynthesis in the gut could be relevant to the improvement and pathogenesis of the GVHD, respectively [77].

There might be intricate innate or adaptive immune mechanisms in the mucosa of gastrointestinal tract. Both innate and adaptive immune mechanisms are integrated with the active phase of gut pathologies [78]. In addition, the immune system might link the gut microbiota even to the progress of neuropsychiatric disorders such as depressive-behaviors [79]. Specific gut microbiota metabolites could alter the Treg/Th17 ratio [80]. In addition, the imbalance of the Treg/Th17 ratio has been implicated in the development of chronic stresses [81]. There might be a probable crosstalk between adaptive immune system and the gut microbiota raising a gut-immune axis. (Figure 3)

## 5. Future perspectives

FMT is known for its outstanding efficacy in recurrent *Clostridioides difficile* infection with more than 90% rate of cure [82], which may aim to restore gut homeostasis by transferring gut bacteria and microbes from healthy individuals' stool [83]. FMT in patients with GVHD has also been associated with favorable clinical outcomes [84]. The treatment seems to be generally safe, but serious adverse events sometimes such as pneumonia and/or sudden death have been reported [85]. At present, the long-term safety remains unclear [86, 87]. As a matter of fact, the detailed effect of modifications in gut microbiota on the disease processes of GVHD in HSCT has not been well understood. However, it has been revealed that protecting the intestinal microenvironment could be a novel strategy to manage a GVHD [88]. Even if it remains unclear whether the effect of modifications of microbiota are indirect to the severity of GVHD or not, they may be advantageous for the

prevention and/or therapy of GVHD in HSCT recipients. Immunologically, it has been shown that a disturbed association between intestine-epithelial cells and the gut microbiota could cause pathogenic responses to the host <sup>[89]</sup>. For example, a high-calorie diet can potentially rearrange the gut microbiota, which could disturb the balance of Th17/Treg cells, interrupt the immunological homeostasis, then exacerbate an inflammatory damages <sup>[90]</sup>. Several human studies have revealed loss of diversity of gut microbiota following the allogeneic HSCT may be linked to the considerable gut injury <sup>[91]</sup>. In addition, prolonged antibiotic use may also decrease the diversity, which might increase the risk of GVHD <sup>[92]</sup>. These findings may indicate potential changeable targets to decrease the risk of GVHD and/or to increase the safety survival rate after allogeneic HSCT. The allogeneic HSCT is a solid potential therapeutic option for patients with a diversity of malignancies. Therefore, further detailed studies will be mandatory to define the immune responses controlled by gut microbiota involved in the development of inflammatory severe responses after allogeneic HSCT. <sup>2</sup> A deeper investigation into internal molecular mechanisms and/or immune-neurological pathways should be performed in the future.

## **CONCLUSION**

<sup>7</sup> Probiotics and/or fecal microbiota transplantation might have a potential efficacy for the treatment of GVHD after hematopoietic stem cell transplantation.

15%

SIMILARITY INDEX

---

PRIMARY SOURCES

---

- |   |                                                                                                                                                                                                |               |
|---|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| 1 | <a href="http://www.aimspress.com">www.aimspress.com</a><br>Internet                                                                                                                           | 82 words — 3% |
| 2 | <a href="http://onlinelibrary.wiley.com">onlinelibrary.wiley.com</a><br>Internet                                                                                                               | 56 words — 2% |
| 3 | <a href="http://www.ncbi.nlm.nih.gov">www.ncbi.nlm.nih.gov</a><br>Internet                                                                                                                     | 50 words — 2% |
| 4 | <a href="http://www.researchgate.net">www.researchgate.net</a><br>Internet                                                                                                                     | 50 words — 2% |
| 5 | Kudakwashe Mhandire, Komalpreet Saggu, Nataliya Prokopenko Buxbaum. "Immunometabolic Therapeutic Targets of Graft-versus-Host Disease (GvHD)", Metabolites, 2021<br>Crossref                   | 35 words — 1% |
| 6 | <a href="http://www.tandfonline.com">www.tandfonline.com</a><br>Internet                                                                                                                       | 28 words — 1% |
| 7 | <a href="http://www.mdpi.com">www.mdpi.com</a><br>Internet                                                                                                                                     | 25 words — 1% |
| 8 | Takehito Asakawa, Michio Onizawa, Chikako Saito, Rie Hikichi et al. "Oral administration of d-serine prevents the onset and progression of colitis in mice", Journal of Gastroenterology, 2021 | 20 words — 1% |

9	<a href="https://api.intechopen.com">api.intechopen.com</a> Internet	17 words — 1%
10	<a href="https://www.hindawi.com">www.hindawi.com</a> Internet	15 words — 1%
11	<a href="https://doaj.org">doaj.org</a> Internet	14 words — < 1%
12	<a href="https://www.jimmunol.org">www.jimmunol.org</a> Internet	14 words — < 1%
13	<a href="https://digital.library.adelaide.edu.au">digital.library.adelaide.edu.au</a> Internet	13 words — < 1%
14	<a href="https://downloads.hindawi.com">downloads.hindawi.com</a> Internet	13 words — < 1%
15	Stefanie Haase, Nicola Wilck, Markus Kleinewietfeld, Dominik N. Müller, Ralf A. Linker. "Sodium chloride triggers Th17 mediated autoimmunity", Journal of Neuroimmunology, 2019 Crossref	12 words — < 1%
16	<a href="https://jeccr.biomedcentral.com">jeccr.biomedcentral.com</a> Internet	12 words — < 1%

EXCLUDE QUOTES ON  
EXCLUDE BIBLIOGRAPHY ON

EXCLUDE SOURCES OFF  
EXCLUDE MATCHES < 12 WORDS