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Research Update (Minireview)

Gut Microbiota in Graft-versus-Host Disease

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Gut microbiota inhabit the host gastrointestinal (GI) tract and play roles in many aspects of metabolic and immunologic homeostasis. Understanding about gut microbiota composition in health and disease is accumulating with the advances in gene sequencing technology. Graft-versus-host disease (GVHD) is a major complication after allogenic bone marrow transplantation (allo-BMT), a gold standard clinical procedure to treat hematologic disorders such as leukemia and lymphomas. Recent studies have shown that a disturbance in the gut microbiota affects GVHD prognosis. Decrease in a compositional diversity is suggested as an independent predictor of GVHD and colonization of noncommensal *Enterococcus* is shown to be involved in unpleasant treatment outcomes. This article describes current understanding about allo-BMT-induced gut microbiota changes and its involvement in the incidence of GVHD. In addition, several putative therapeutic strategies to decrease GVHD-related mortality after allo-BMT are discussed.

Key Words: Gut microbiota, allo-BMT, GVHD, *Enterococcus* domination, VRE

INTRODUCTION

The human gut harbors trillions of microorganisms, which are referred to the gut microbiota, play a fundamental role in human health and disease. Interactions between gut microbiota and the enterocytes provide structural, metabolic, and immunologic supports in the host gastrointestinal (GI) tract (1). However, disturbance in the normal gut microbiota occurs in many pathological conditions including hematologic disorders such as leukemia, aplastic anemia, and lymphomas (2). Allogenic bone marrow transplantation (allo-BMT) is considered as a standard therapy for the treatment of hematologic disorders. However, the success of the treatment is often hindered by the incidence of graft-versus-host disease (GVHD) that donor T cells recognize the host organ as foreign and induce damage to the GI tract, liver, and skin. Studies performed in early 1970s reported that allo-BMT in germ-free mice or antibiotics administration could increase survival without symptoms of GVHD (3, 4). These had been further explored in allo-BMT patients and showed that antibiotics treatment and maintaining near-sterile environment could effectively decreased GVHD-related mortality (5, 6). Nevertheless, characterization of human gut microbiota involved in the incidence of GVHD has been limited by culture-based microbiological techniques hence development of supplemental remedy targeting gut microbiota was still clinically unavailable. Recently, advances in genome

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sequencing technology and bioinformatics aid our understanding about the human microbiota in health and disease (7). In this regard, recent studies have re-addressed a relationship between gut microbiota and GVHD incidence using bacterial 16S ribosomal RNA (rRNA) gene sequencing (8~10). In this review, I described current understanding about gut microbiota composition changes in GVHD and pathophysiological involvement of enterococcal domination in GVHD-related mortality. In addition, potential therapeutic strategies to modify gut microbiota were suggested.

Gut microbiota composition and GVHD-related mortality

Under normal conditions, commensal bacteria produce antimicrobial peptides to maintain the compositional diversity and immunomodulatory function of the gut microbiota (1). Disturbance in the microbiota composition could occur during the process of allo-BMT that comprises bone marrow ablation by radiotherapy or chemotherapy, antibiotics treatment, and damage in mucosal barrier due to GVHD. Evidence suggests that microbiota diversity is an independent determinant of the incidence of GVHD following allo-BMT. Jenq *et al*. have analyzed bacterial 16S rRNA gene copies and sequence in a mouse model of allo-BMT and GVHD (8). Although bacterial density has increased upon transplantation, the diversity is significantly decreased in GVHD-induced mice. This was confirmed in human stool specimen which showed a loss of microbiota diversity after onset of GVHD in allo-BMT patients. In addition, microbiota diversity before allo-BMT is reported as a potential indicator of GVHD incidence (9). Taur *et al*. divided 80 patients undergoing allo-BMT into three groups according to the microbiota diversity and have shown that patients who were classified as a low diversity group showed a higher mortality rate. In contrast, with the patients who harbors high or intermediate microbiota diversity showed 60~67% overall survival rate until 3 years after the transplantation.

A dramatic shift in the major composition of microbiota occurs with the onset of GVHD. Normal gut microbiota consists of mainly Bacteroidetes and Firmicutes. Firmicutes comprises more than 80% of stool microbiome. Among the phylum Firmicutes, Clostridia (*Clostridium* and *Eubacterum*) constitutes the majority of the normal microflora, but Bacilli (*Lactobacillus* and *Enterococcus*) increases dramatically in GVHD (8, 10, 11). It is also suggested that if a relative abundance of *Enterococcus*, which is almost absent in normal microflora, exceeds 20% of total microbiota in patient's stool specimen, the risk of GVHD frequency increases (10). Use of broad-spectrum antibiotics seems to be involved in an increase in enterococci susceptibility while reducing survival of other commensal Firmicutes (10, 11). For example, metronidazole administration increased enterococcal domination 3-fold and this was shown to increase risk of vancomycin-resistant *Enterococcus* (VRE) bacteremia 9-fold (11). VRE infection in GVHD pathogenesis is discussed in the following section in more detail.

In another study, it is demonstrated that patients harboring a great abundance of Lachnospiraceae are likely to show better survival after allo-BMT (9). A subsequent study has revealed that abundance of *Blautia* significantly reduces GVHD lethality and increases overall survival (12). Anti-inflammatory effect of *Blautia* may involve induction of regulatory T cell infiltration into the intestine which has been achieved by the administration of selected mixture of Clostridia (13). Another possible mechanism of action of Lachnospiraceae in mitigation of GVHD is via producing butyrate (14). Microbial metabolites profiling after allo-BMT revealed a significant decrease in the amount of butyrate in intestinal tissue compared to the normal or syngeneic BMT group of mice. Reduced synthesis of butyrate, a histone deacetylase inhibitor (HDACi), resulted in decrease in histone H4 acetylation in intestinal epithelial cells (IECs) which is associated with apoptotic cell death and GVHD. Conversely, exogenous administration of butyrate mitigated GVHD by reducing IEC apoptosis via enhancing IEC junctional integrity (14). Function of butyrate on Fas-mediated T cell apoptosis was reported (15) which could be further evaluated in GVHD. Jenq *et al*. have suggested that use of antibiotics against anaerobic bacteria or prolonged total parenteral nutrition is involved in loss of *Blautia* (12). Therefore, judicious selection of antibiotics has to be practiced in the hospital and promotion of enteral nutrition after allo-

BMT could be encouraged to preserve normal microbial metabolites. If necessary, modulation of microbial metabolites could be considered as an option for relieving GVHD severity.

Enterococcus colonization in GVHD pathophysiology

Enterococci generally are considered as nonpathogenic commensal bacteria displaying low levels of virulence. However, their inherent characteristics exaggerate the enterococci domination as an outstanding clinical problem (16). First of all, they have an ability to resist antimicrobial agents and disseminate vancomycin resistance gene clusters. These enable multi-resistant nosocomial pathogens to colonize in patients and lead to VRE infection fatal without effective antimicrobial therapy. Secondly, enterococci can survive for long periods on surfaces and tolerate harsh environmental conditions such as heat, chlorine, and alcohol. Strain-specific PCR combined with 16S rRNA analysis revealed a significant increase in *E. faecalis* and *E. faecium* in patients with GVHD (10). Those two *Enterococcus* strains are known to cause hospital-associated enterococcal infections, which dramatically increased with a use of vancomycin and broad-spectrum antibiotics since the late 1970s (16), suggesting *Enterococcus* domination may be a core risk factor for GVHD-related mortality.

Enterococci produce varieties of virulence factors that are involved in infections in abdominal cavity, endocardium, and urinary tract (17). Especially for the hospital-acquired isolates, *E. faecalis* and *E. faecium*, distinctive expressions of several molecules are involved in the pathogenesis. Firstly, enterococci colonization can take place through the expression of Enterococcal surface protein (esp) (18), PilA, and PilB (19). Those are involved in host cell wall anchoring and biofilm formation. Interactions between several enterococcal microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and host cell extracellular matrix proteins such as collagen type V and fibrinogen also facilitates colonization of *Enterococcus* (17, 20). Next, metalloprotease gelatinase E (GelE) production from *E. faecalis* compromise epithelial barrier function by degrading E-cadherin and this contributes to intestinal inflammation (21).

Reduction in the production of antimicrobial peptide, RegIIIγ associates VRE infection (22). Gram-negative commensal bacteria inhibit colonization of VRE by triggering RegIIIγ production from intestinal epithelial cells and Paneth cells. However, use of broad-spectrum antibiotics inhibits the growth of Gram-negative bacteria and results in the overgrowth of enterococci (23). Restoration of RegIIIγ production by lipopolysaccharides (LPS) (23) or flagellin administration (24) suggests a therapeutic potential of TLR agonists on regulation of *Enterococcus* colonization after allo-BMT.

Therapeutic strategies to modify gut microbiota

As discussed above, loss of gut microbiota diversity and colonization of *Enterococcus* are driving factors of GVHD-related mortality in allo-BMT patients. Accumulating evidence suggest that several strategies could be trialled in steps of allo-BMT to maintain microbiota diversity and prevent enterococcal domination. A study has demonstrated that microbiota diversity before allo-BMT impacts treatment outcomes (9). Thus, examination of patient's stool specimen could be performed to determine the timing for allo-BMT, especially for the patients who underwent antibiotics administrations. Alternatively, modulation of gut microbiota by introducing beneficial commensal bacteria could be considered to improve the medical prognosis. As studies have shown that reintroduction of *Lactobacillus johnsonii* after BMT increased survival by inhibiting *Enterococcus* expansion (8) and administration of Clostridia ameliorated colitis (13).

Prevention of hospital-acquired *Enterococcus* infections could be attained from the hospital care units. Maintaining near-sterile environment has to be achieved as the first line of defense against *E. faecalis* and/or *E. faecium* infections. Hospitalization of patients in a laminar air flow room before and after allo-BMT (5) could be considered and a strict hygienic management has to be performed. Given broad-spectrum antibiotic administration increases VRE susceptibility (11), selection of antibiotics has to be practiced with caution. Further identification of genera involved in GVHD-related morality followed by a re-affirmation across BMT

centers would increase our understanding and support a development of therapeutics to modulate gut microbiota.

CLOSING REMARKS

Gut microbiota maintain metabolic and immunologic homeostasis of the host GI tract while dysbiosis often results in a fatal outcomes such as GVHD in allo-BMT patients. Here I have summarized current understanding about compositional changes of gut microbiota in GVHD-related mortality, possible inducers of enterococci colonization, and therapeutic strategies to defense against noncommensal bacteria expansion. Although those data has to be further verified from different BMT centers in different nations as gut microbiota is subject to be changed by diet, current evidence suggests a potential to develop novel therapeutics aiming at modifying gut microbiota.

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