High-choline diets ameliorate acute graft-versus-host disease

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is one of the most effective methods in the treatment of leukemia and other malignant hematological diseases. However, the occurrence of acute graft-versus-host disease (aGVHD) seriously impairs the survival and prognosis of patients, and the mortality rate of aGVHD is about 10%.[1] During the development of aGVHD, the initial step consists of injury to the host tissues caused by conditioning regimen. Increasing reports have indicated that gastrointestinal toxicity, in turn, could aggravate aGVHD through modulating the severity of intestinal inflammation. The gut mucosal damage mediated by aGVHD causes the bacterial translocation which further stimulates antigen presenting cells thereby upregulates the production of pro-inflammatory cytokines such as interleukin-1 (IL-1) and interleukin-6 (IL-6).[2] This proinflammatory process provides appropriate environment for T-cell allo-activation and has been proposed to play a particularly critical role in the initial stage of aGVHD. In addition, the alterations in the compositions of intestinal microbiota after allo-HSCT has also been shown to be closely associated with the onset of aGVHD. The loss of microbial diversity can result in the relative abundance of pathogenic bacteria and the loss of Clostridia species, which is considered to be benefit for the proliferation and activation of regulatory T cells (Treg cells).[3] This imbalance of the gut microbiota finally breaks the immune homeostasis and promotes the development of aGVHD.

Besides the loss of bacterial diversity and alteration of bacterial composition, the variety of microbial metabolites after allo-HSCT has also been shown to play an important role in aGVHD. Clostridial plays an important role in anti-inflammatory homeostasis, including promoting intestinal epithelial cells (IECs) to secret TGF-β through the production of butyrate, thus to expand and activate Treg cells in intestinal tract and inhibit the inflammatory effect of aGVHD.[3] In addition, butyrate can mitigate aGVHD through modulating histone acetylation in IECs, reducing apoptosis and improving the junctional integrity of IECs. Moreover, the infusion of clostridiales isolates mixture with high concentration of butyrate can prolong the survival of aGVHD mice.[4] Although most studies to date have elucidated the effect of butyrate on aGVHD development, the microbiota metabolite diversity is much more complex than the diversity of microbiota,[5] and numerous complexities of aGVHD-metabolite remain to be explicated.

In a recent study published in Blood, Kunpeng Wu *et al* have demonstrated that either choline-metabolized trimethylamine N-oxide (TMAO) or high choline diet ameliorate the development of aGVHD by promoting the differentiation and proliferation of pathogenic donor T cells (Th1 and Th17), which is resulted from the effects of TMAO on the shifting macrophages-immunity toward F4/80$^+$CD11b$^+$CD16/32$^+$M1 macrophages through NLRP3 inflammasome activation.[6]

Extensive research has shown that diet is an important modulator of the metabolites production of the gut microbiota. Studies reported that Choline, phosphatidylcholine and carnitine-containing dietary can generate TMAO by the altered action of gut microbiota.[5] Increased levels of plasma TMAO was identified as an independent risk factor for promoting atherosclerosis, which induced vascular inflammation through NLRP3 inflammasome activation.[7] Although previous studies have indicated that TMAO may play an important role in pathological or inflammatory conditions, Kunpeng Wu et al are the first to clarify its immunoregulatory role in the pathogenesis of aGVHD. They found that oral TMAO treatment significantly accelerated aGVHD, whereas 3,3-dimethyl-1-butanol (DMB), the analog of choline, mediated protective effect in aGVHD. It is well-known that microbial metabolite mainly influenced the development and polarization of T lymphocytes.[8] Correspondingly, Kunpeng Wu et al showed that the differentiation of Th1 and Th17 was remarkably upregulated in TMAO-treated GVHD recipients through M1 macrophage polarization. Based on the rationale that NLRP3 inflammasome activation is critical for M1 macrophage polarization and TMAO could promote cellular NLRP3 inflammasome activation in endothelial cells, Kunpeng Wu et al extended their study to demonstrate the effects of NLRP3 inflammasome activation on M1 polarization in aGVHD with TMAO treatment. Notably, an NLRP3 inhibitor (CY-09), as well as Nlrp3$^{-/-}$ BMDMs attenuated TMAO-induced

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polarization of M1. They investigated that TMAO obviously stimulated reactive oxygen species (ROS) generation, in particular, mitochondrial ROS (mtROS), which was the key player during the activation of the NLRP3 inflammasome.[9] They additionally found that TMAO promoted NLRP3 expression by inducing NF-κB nuclear localization. Finally, they showed that TMAO was not able to exacerbate aGVHD, nor did it induce M1 polarization when NLRP3 was lacking, indicating TMAO might contribute to aGVHD by activating the NLRP3 inflammasome, similar to its function in atherosclerosis.

Since dentritic cells (DCs) are important antigen presenting cells (APCs) for initiation of pathogenic donor T-cell differentiation during aGVHD,[10] additional studies are needed to determine the effects of TMAO on the expansion, function and activation of DCs. Moreover, whether macrophages play a critical role during TMAO-induced acceleration of aGVHD is also needed to be further confirmed through in vivo experiments, such as evaluating the impact of macrophages clearance on TMAO-mediated aGVHD exacerbation. Given that elevated blood levels of TMAO are shown to be closely associated with atherosclerosis in human,[7] additional studies are needed to determine whether plasma TMAO levels in allo-HSCT patients are also linked to the severity of aGVHD, and whether high plasma TMAO levels could predict aGVHD risk. Furthermore, utilizing humanized aGVHD mouse model or in vitro cell experiments to detect the effects of TMAO on human T cell differentiation may add to preclinical evidence that blockage of TMAO pathway offers promise as a therapy to prevent aGVHD in humans. It will also be critical to determine the effects of TMAO blockade on graft-versus-leukemia (GVL) effect. It is important to note that M1 macrophages exert anti-tumor effects through secretion of ROS, promotion of Th1 and $CD8^+$T cells response.[11,12] Even though high plasma TMAO levels have been reported to be associated with colorectal cancer (CRC), the causative link between high TMAO concentration and cancer remains unknown.[13] Therefore, one might have concern that TMAO blockade could compromise the GVL effect.

Recent clinical studies demonstrated that fecal microbiota transplantation (FMT), one of the major strategies to intervene intestinal dysbacteriosis, can restore intestinal microbiota diversity and have the potential to reduce aGVHD occurrence.[14] However, part of the patients did not benefit from FMT treatment, and even have a risk for bacterial infection.[15] Therefore, defining pivotal gut microbial metabolites in aGVHD and exploring the interaction between gut microbial metabolites, diet and aGVHD process are essential to provide new ideas for effective and safe therapy during aGVHD. Kunpeng Wu et al have clearly demonstrated that high choline diet generated TMAO can obviously exacerbate aGVHD through promoting the differentiation of Th1 and Th17 cells by activating NLRP3 inflammasome, thus TMAO blockade pathway may represent a novel approach to preventing aGVHD. Nevertheless, it is still important to determine the association between high plasma TMAO levels and aGVHD development in allo-HSCT patients. Meanwhile, further studies are needed to elucidate the impact of TMAO on human T cell differentiation, and the effect of TMAO blockade on GVL responses. The results from these studies will determine whether TMAO blockade could move closer to use in clinic and whether TMAO blockade has the potential to separate aGVHD and GVL effects.

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