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HEMATOPOIESIS AND STEM CELLS

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A gut-graft axis mediated by microbiota

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In this issue of *Blood*, Miltiadous et al report on the association between the early posttransplant gut microbiome and immune cell reconstitution in 894 recipients of an allogeneic hematopoietic cell transplantation (allo-HCT).[1] They examined 2067 stool samples for intestinal microbiota using amplicon sequencing and 2370 peripheral blood samples for immune cell recovery using flow cytometry. The 3 stem cell sources used for the transplants—unmodified bone marrow, unmodified peripheral blood stem cells (PBSCs), and CD34-selected PBSC grafts—were studied separately. The authors found that diversity in fecal microbiota was predictive of CD4$^+$ T-cell counts at 3 months in the CD34-selected cohort. In addition, higher relative abundance of fecal *Staphylococcus* in the early post-HCT period predicted worse subsequent CD4$^+$ T-cell recovery.

Robust reconstitution of donor immune cells is essential for successful clinical outcomes after allo-HCT.[2] Delay in immune reconstitution is associated with increased risk of serious infections and higher mortality.[2,3] In addition, the reconstitution kinetics of distinct immune cell subsets has a significant influence on clinical outcomes. Early recovery of total CD4$^+$ and naïve CD4$^+$ T cells predicts lower risks of infections, chronic graft-versus-host disease (cGVHD), and transplant-related mortality (TRM),[3] whereas rapid reconstitution of regulatory T cells is associated with lower risk of cGVHD. Early recovery of CD8$^+$ T cells after transplantation can reduce the risk of infections and relapse, but it predicts a higher risk of acute GVHD (aGVHD). Rapid recovery of γδ T cells is associated with lower risks of infections and relapse. Early recovery of natural killer cells predicts lower risk of relapse and higher risk of GVHD. Stronger responses to vaccinations with lower rates of infections and TRM have been linked to earlier reconstitution of B cells.

Donor type, graft source, T-cell depletion, and GVHD prophylaxis type are among established factors that can influence immune reconstitution.[2,3] More recently, several preclinical and observational studies have highlighted the potential effect of intestinal microbiota on immune reconstitution after transplantation.[4,5] Exposure to antibiotics, changes in nutrition, and conditioning-related impairment of the gut barrier all cause damage to the gut microbiota. This damage can be measured in various ways, including α diversity, abundance of specific taxa, microbial network structures, community-level metabolic function, and antibiotic-resistant gene content. When patients are referred for allo-HCT, their gut microbiota is often already perturbed,[5] a finding that can be traced back to when patients received chemotherapy for their underlying hematologic disease.[6] Unresolved previous injuries are worsened during allo-HCT in many patients, resulting in mono- or oligo-dominated communities with low diversity at the time of engraftment.[5] In a large multicenter study, lower diversity of peri-engraftment intestinal microbiota was associated with higher TRM in patients receiving a T-cell replete graft.[5]

Although these studies indicate a strong association between the diversity of microbiota and transplantation outcomes, the presence and nature of a causal link have remained obscure. TRM can be the result of various causes including infection, organ failure, and GVHD. Indeed, decreased diversity of peri-engraftment intestinal microbiota predicts higher risk of aGVHD and its associated mortality.[7] The potential contribution of microbiota injury to each of the various causes of TRM is unknown. There is a mutual interaction between the immune system and the gut microbiota. The gut microbiota can modulate cytokine release from immune cells,[8] and microbiota-derived metabolites contribute to steady-state granulopoiesis. In return, the immune cells can modulate the mucosal bacteria by immunoglobulin A. In a murine model, antibiotic therapy resulted in delayed lymphocyte and granulocyte reconstitution after allo-HCT because of reduced sucrose production by the gut microbiota.[4] Sucrose supplementation led to stimulation of hematopoiesis and improved neutrophil recovery.

Importantly, Miltiadous et al adjusted their analysis for several potential confounders including age, graft source, GVHD prophylaxis, cytomegalovirus reactivation, exposure to anti-thymocyte globulin, and use of high-dose steroids. One question that can be explored further is the potential contribution of antibiotics to microbiota diversity, composition, and count recovery. Specifically, could the observed association between microbiota and immune reconstitution be mediated or even confounded by

antibiotics? Some antibiotics commonly used in the setting of allo-HCT (eg, linezolid) can suppress hematopoiesis and *Staphylococcus*. In a previous study,[9] the abundance of *Staphylococcus* was positively associated with lymphocyte counts obtained from routine complete blood cell counts. The Miltiadous et al study used more granular data on immune cell subsets from flow cytometry. The observation that *Staphylococcus* was associated with a worse reconstitution of $CD4^+$ T cells in their study but higher total lymphocyte counts in the previous study is worth further research. Does this reflect a potential positive impact of *Staphylococcus* on other lymphocyte subsets not evaluated in their current study? What is the function and clinical impact of those subsets? Different species and strains of the same genus often have different physiology and effects on the host. Such effects cannot be reliably inferred from genus-level data generated from amplicon sequencing.

What should the path forward be and how could the findings in this study improve clinical practice? Microbial community-level interventions such as transplantation of fecal microbiota can restore the diversity of the microbiota and eradicate antibiotic-resistant genes.[10] However, the emergent properties of the new communities (potentially with different network structures and composition) in relation to host physiology and HCT outcomes are largely unknown. Precisely defined microbiota interventions (eg, single taxon probiotics) try to restore a specific beneficial taxon within a large community of bacteria that has undergone major disruptions. However, it remains unknown whether the impact (if any) of such interventions is clinically meaningful. Nonetheless, alternative methods such as shotgun sequencing can be attempted in follow-up studies to delineate specific species and strains within the same promising genera that may mediate microbiota associations with immune reconstitution. Ultimately, it seems important to validate findings in other centers using different antibiotic and non-antibiotic practices before clinical therapeutics can be developed.

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Nakamura et al, page 2770

(SUMO)-wrestling with rituximab

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In this issue of *Blood*, Nakamura et al show that the SUMOylation inhibitor subasumstat (TAK-981) augments the ability of rituximab to evoke macrophage phagocytosis and natural killer (NK) cell cytotoxicity and delivers improved antitumor activity in murine models (see figure).[1] Anti-CD20 monoclonal antibodies (mAbs) have become the mainstay of treatment for B-cell disorders ranging from non-Hodgkin lymphoma to rheumatoid arthritis and multiple sclerosis, with next-generation mAbs building on the success of rituximab.[2] Nevertheless, treatment success is varied, and the challenge is to overcome resistance and improve outcomes for patients.[3] Nakamura et al present one such approach.

SUMOylation[4] represents a posttranslational protein modification akin to ubiquitination that controls multiple cellular and organismal processes, including inflammation, whereupon a small ubiquitin-related modifier (SUMO) is added and removed dynamically from relevant target proteins to modulate their activity.

The study by Nakamura et al uses the SUMOylation inhibitor subasumstat to promote the innate antitumor immune response through the induction of type I interferons (IFN1s). By using human and mouse macrophages, they convincingly show the induction of IFN1s after treatment with TAK-981 alongside other inflammatory mediators. This activity was sufficient to elicit inflammatory M1-type macrophage skewing and was largely dependent on IFN1s. TAK-981 also impaired anti-inflammatory M2-type polarization, suppressing interleukin-4 (IL-4)–dependent M2 induction either when TAK-981 was added simultaneously or when IL-4 signaling had already been