REFERENCES

1. Berger G, Kroeze LI, Koorenhof-Scheele TN, et al. Early detection and evolution of pre-leukemic clones in therapy-related myeloid neoplasms following autologous SCT. *Blood*. 2018;131(16):1846-1857.
2. Smith SM, Le Beau MM, Huo D, et al. Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. *Blood*. 2003;102(1):43-52.
3. Takahashi K, Wang F, Kantarjian H, et al. Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. *Lancet Oncol*. 2017;18(1):100-111.
4. Gillis NK, Ball M, Zhang Q, et al. Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. *Lancet Oncol*. 2017;18(1):112-121.
5. Coombs CC, Zehir A, Devlin SM, et al. Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. *Cell Stem Cell*. 2017;21(3):374-382.e4.
6. Takahashi K, Wang F, Kantarjian H, et al. Copy number alterations detected as clonal hematopoiesis of indeterminate potential. *Blood Adv*. 2017;1(15):1031-1036.
7. Wong TN, Ramsingh G, Young AL, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. *Nature*. 2015;518(7540):552-555.
8. Gibson CJ, Lindsley RC, Tchekmedyian V, et al. Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. *J Clin Oncol*. 2017;35(14):1598-1605.
9. Young AL, Challen GA, Birmann BM, Druley TE. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. *Nat Commun*. 2016;7:12484.
10. Shlush LI, Zandi S, Mitchell A, et al; HALT Pan-Leukemia Gene Panel Consortium. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia [published correction appears in Nature. 2014;508(7496):420]. *Nature*. 2014;506(7488):328-333.

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TRANSPLANTATION

Comment on Hülsdünker et al, page 1858

Pathogenic neutrophils in acute GVHD

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In this issue of *Blood*, Hülsdünker et al show how recipient neutrophils contribute to the pathogenesis of acute graft-versus-host disease (GVHD), a complication of allogeneic hematopoietic cell transplantation (HCT) caused by donor T cells that recognize recipient alloantigens.[1]

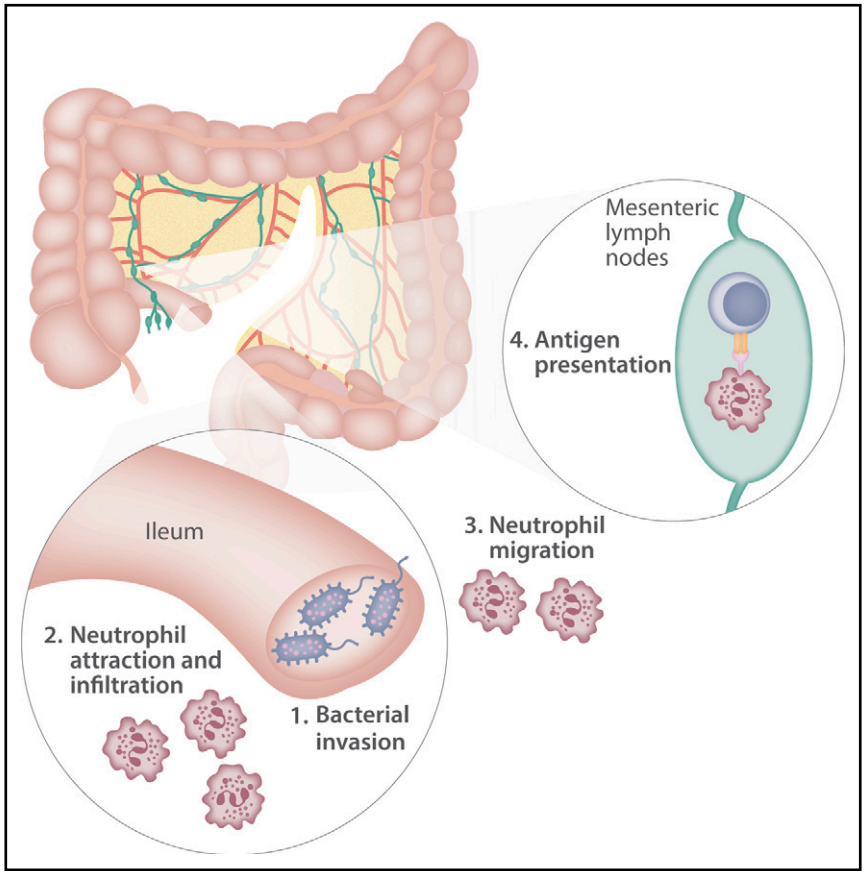
Previous studies of GVHD have shown that many types of hematopoietic cells can present recipient alloantigens to donor T cells.[2] These cell types include dendritic cells, plasmacytoid dendritic cells, macrophages, B cells, and Langerhans cells. In addition, certain recipient cells outside the hematopoietic lineage can present alloantigens that cause GVHD. Neutrophils are known to present antigens to T cells under pathological or inflammatory conditions,[3] but Hülsdünker et al are the first to investigate their unexpected role in the pathogenesis of acute GVHD.

As illustrated in the figure, they showed that intestinal bacterial preferentially invaded the ileal mucosa after damage caused by total body irradiation. As part of the inflammatory response to bacteria, neutrophils infiltrated the ileal mucosa and were activated to express MHC class II molecules. In the ileum, neutrophils expressing a transgenic photoconversion reporter were labeled by light exposure, and their subsequent migration was traced to draining mesenteric lymph nodes. In lymph nodes, the activated neutrophils presented alloantigen to donor T cells that contributed to the development of acute GVHD. Consistent with this hypothesis, antibody-mediated depletion of neutrophils in the recipient on the day before HCT decreased the severity of acute GVHD.

Based on the rationale that granulocyte colony-stimulating factor (G-CSF) signals through Janus kinase 1 (JAK1) to stimulate neutrophil differentiation, Hülsdünker et al extended their study to evaluate the effects of the JAK1/2 inhibitor ruxolitinib on antigen presentation by neutrophils after HCT. Ruxolitinib inhibited the activation-induced expression of MHC class II molecules by neutrophils and prevented their migration from the ileum to mesenteric lymph nodes. Ironically, neutrophils are induced to express MHC class II molecules by interferon γ (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-3 (IL-3), but not by G-CSF,[3] and while IFN-γ signals through JAK1, GM-CSF and IL-3 signal through JAK2.

Drug-based approaches for controlling GVHD after allogeneic HCT in humans have focused primarily on inhibition of T-cell responses with the use of antimetabolites such as methotrexate or mycophenolate mofetil, calcineurin inhibitors such as cyclosporine and tacrolimus, mechanistic target of rapamycin inhibitors such as sirolimus, and more recently, the alkylating agent cyclophosphamide.[4] Observations that type II cytokine receptors activate adaptive T-cell responses through JAK-mediated phosphorylation of signal transducers of activation and transcription (STAT) have prompted preclinical studies testing whether JAK inhibitors could prevent GVHD. One such study showed that administration of ruxolitinib beginning on the day before HCT and continuing until day 20 after HCT decreased the severity of acute GVHD in mice.[5]

The current study showing that neutrophils are involved in the pathogenesis of GVHD adds to evidence that the path to GVHD begins with inflammatory innate immune responses caused by the conditioning regimen before HCT.[4] These innate immune responses facilitate and enhance adaptive donor T-cell immune responses stimulated by recipient alloantigens that are presented redundantly by a wide variety of cell types. The current study also adds to preclinical evidence that inhibition of JAK-STAT signaling offers promise as a way to prevent GVHD in humans not only through effects on adaptive T-cell responses,[6] but also through effects on innate immune responses. For example, ruxolitinib inhibits antigen presentation not only by neutrophils, but also by dendritic cells and monocyte-derived dendritic cells.[7,8] Additional studies are needed to determine whether JAK inhibitors have similar effects on other types of cells involved in antigen presentation. Even



Damage caused by the pretransplant conditioning regimen allows luminal bacteria to invade the ileal mucosa. Recipient neutrophils attracted as part of the inflammatory response infiltrate the ileal mucosa and upregulate expression of major histocompatibility complex (MHC) class II molecules. Activated neutrophils then migrate to draining lymph nodes where they present recipient alloantigens to donor T cells. Activated donor T cells subsequently traffic to target tissues and cause GVHD. Professional illustration by Somersault18:24.

though it is not feasible to prevent GVHD by depleting all recipient and donor-derived antigen-presenting cells, it may be possible to identify a limited set of critical signaling pathways that are widely involved across the various cell types that present alloantigens.

JAK inhibitors could be used during a limited time window early after HCT in ways that allow subsequent immune reconstitution and graft-versus-leukemia effects. The potential success of such an approach has already been demonstrated not only by preclinical studies of ruxolitinib, but also by preclinical[9] and clinical[10] evidence that the severity of acute GVHD can be attenuated by neutralization of IL-6, a cytokine that signals through JAK1. Before embarking on clinical trials testing the use of JAK inhibitors to prevent GVHD in humans, we need evidence that JAK inhibition will not interfere with the ability of hematopoietic stem cells to repopulate the marrow or the ability of donor T cells to prevent graft rejection and eliminate malignant cells that survive the pretransplant conditioning regimen. Additional work will be needed in order to determine the optimal dose and duration of treatment.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Hülsdünker J, Ottmüller KJ, Neeff HP, et al. Neutrophils provide cellular communication between ileum and mesenteric lymph nodes at graft-versus-host disease onset. *Blood*. 2018;131(16):1858-1869.
2. Koyama M, Hill GR. Alloantigen presentation and graft-versus-host disease: fuel for the fire. *Blood*. 2016;127(24):2963-2970.
3. Lin A, Loré K. Granulocytes: new members of the antigen-presenting cell family. *Front Immunol*. 2017;8:1781.
4. Zeiser R, Blazar BR. Acute graft-versus-host disease—biologic process, prevention, and therapy. *N Engl J Med*. 2017;377(22):2167-2179.
5. Spoerl S, Mathew NR, Bscheider M, et al. Activity of therapeutic JAK 1/2 blockade in graft-versus-host disease. *Blood*. 2014;123(24):3832-3842.
6. Betts BC, Abdel-Wahab O, Curran SA, et al. Janus kinase-2 inhibition induces durable tolerance to alloantigen by human dendritic cell-stimulated T cells yet preserves immunity to recall antigen. *Blood*. 2011;118(19):5330-5339.
7. Heine A, Held SAE, Daecke SN, et al. The JAK-inhibitor ruxolitinib impairs dendritic cell function in vitro and in vivo. *Blood*. 2013;122(7):1192-1202.
8. Stickel N, Hanke K, Marschner D, et al. MicroRNA-146a reduces MHC-II expression via targeting JAK/STAT signaling in dendritic cells after stem cell transplantation. *Leukemia*. 2017;31(12):2732-2741.
9. Tawara I, Koyama M, Liu C, et al. Interleukin-6 modulates graft-versus-host responses after experimental allogeneic bone marrow transplantation. *Clin Cancer Res*. 2011;17(1):77-88.
10. Kennedy GA, Varelias A, Vuckovic S, et al. Addition of interleukin-6 inhibition with tocilizumab to standard graft-versus-host disease prophylaxis after allogeneic stem-cell transplantation: a phase 1/2 trial. *Lancet Oncol*. 2014;15(13):1451-1459.

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