

Comment on Piper et al, page 568

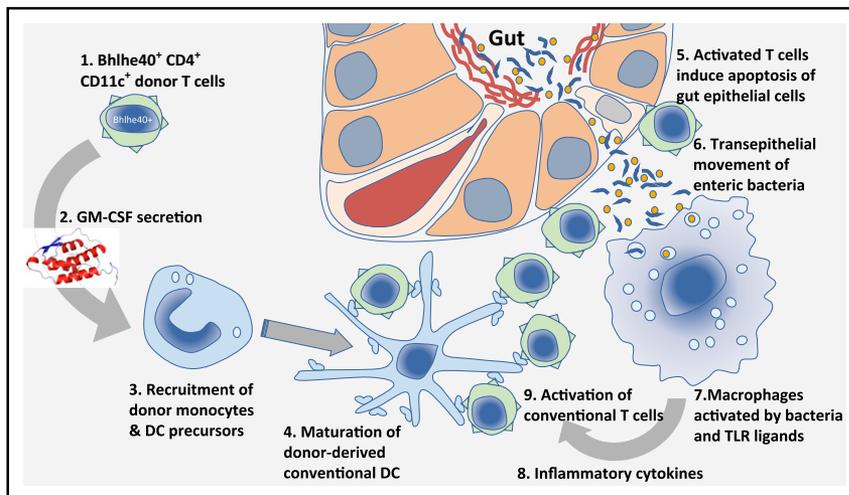
# A new role for an old cytokine: GM-CSF amplifies GVHD

Edmund K. Waller | Emory University

**In this issue of *Blood*, Piper et al find that donor T cells that secrete granulocyte-macrophage colony-stimulating factor (GM-CSF) promote graft-versus-host disease (GVHD) by recruiting donor dendritic cells.<sup>1</sup> This amplifies the activation of alloreactive T cells and increases the severity of GVHD. The authors identified GM-CSF secretion in a rare population of CD11c<sup>+</sup> CD4<sup>+</sup> T cells that express the transcription factor Bhlhe40. Piper et al showed that Bhlhe40<sup>+</sup> CD4<sup>+</sup> donor T cells are central to the development of GVHD in the gut in murine models of allogeneic bone marrow transplantation (BMT). Transplantation of either GM-CSF or Bhlhe40 knockout donor T cells resulted in significantly lower incidence of GVHD in allogeneic BMT recipients. This paper is of broad interest to hematologists and immunologists as it illuminates the role of donor T cells in activating dendritic cells and positions GM-CSF-producing T cells as a critical link between innate and adaptive immune responses.**

The physiological role for GM-CSF has been unclear. GM-CSF was the first colony-stimulating factor to enter clinical trials and is Food and Drug Administration approved for treatment of neutropenia after chemotherapy, stem cell transplantation, graft failure, or stem cell mobilization.<sup>2</sup> In clinical practice, the use of GM-CSF in neutropenic patients and stem cell mobilization has largely been supplanted by granulocyte

colony-stimulating factor, another hematopoietic cytokine that promotes granulocyte development. However, GM-CSF is dispensable for steady state hematopoiesis in knockout mice.<sup>3</sup> GM-CSF knockout mice have normal blood counts and differentiation of hematopoietic stem cells along the myeloid lineage. The major differentiating clinical feature of GM-CSF knockout mice is alveolar proteinosis: the accumulation of



Bhlhe40<sup>+</sup> CD4<sup>+</sup> donor T cells secrete GM-CSF that enhances indirect alloantigen presentation by dendritic cells amplifying GVHD in allogeneic hematopoietic stem cell transplant recipients. The sequence of events in the pathway proposed by Piper et al is shown with local production of GM-CSF (2) by donor T cells in the interstitium of the gut recruiting monocytes (3) that are matured into conventional dendritic cells (4), leading to activation of donor T cells primed to allo-antigens that cause apoptosis of gut epithelial cells (5), leakage of gut microbes into interstitium (6), activation of macrophages that phagocytose bacteria (7), release of inflammatory cytokines (8), and amplification of GVHD by activation of conventional donor T cells (9). DC, dendritic cells; TLR, Toll-like receptor.

lipid and proteinaceous material in the alveoli of the lungs as a consequence of decreased phagocytic activity of macrophages.<sup>4</sup> The findings by Piper et al support emerging data that a key physiological role of GM-CSF is to amplify adaptive immunity and inflammatory responses by recruiting dendritic cells. This in turn facilitates epitope spreading via indirect presentation of peptide antigens and accelerates the activation of conventional T cells (see figure).

The study by Piper et al also addresses a long-standing question in the pathogenesis of gut GVHD: how are alloantigen-specific responses amplified in GVHD-target tissues? The authors describe how transplant recipients received an infusion of donor T cells with a diverse T-cell receptor repertoire. Some of these receptors recognized host peptides derived from minor or major histocompatibility antigens that were directly expressed by host antigen-presenting cells or indirectly expressed by donor-derived dendritic cells recruited to the site of antigen presentation.<sup>5</sup> T cells coexpressing CD4 (a marker on T cells and monocytes) and CD11c (a marker for monocytes and dendritic cells) express Bhlhe40, a transcription factor that regulates GM-CSF synthesis and has been shown to promote neuroinflammation in mice.<sup>6</sup> Bhlhe40<sup>+</sup> donor T cells secrete GM-CSF, leading to recruitment and activation of donor-derived monocytes and dendritic cells, which then cross-present alloantigens derived from gut tissues damaged by the conditioning regimen and inflammatory responses to chemotherapy. The coexistence of activated T cells and dendritic cells cross-presenting minor histocompatibility antigen (mHAg)-derived peptides amplifies alloimmune responses and leads to generalized inflammation, apoptosis of gut epithelial cells, breakdown of the gut epithelial barrier, and extraluminal translocation of enteric bacteria, diarrhea, and blood loss: the clinical manifestations of GVHD. According to Piper et al, the signaling pathway downstream of Bhlhe40 and GM-CSF is independent of proinflammatory cytokines interleukin-1 (IL-1), IL-23, and IL-6. However, higher GM-CSF levels still activate donor-derived classical dendritic cells in the colon to secrete IL-23, which then increases indirect antigen presentation and promotes GVHD. Notably, Bhlhe40 knockout CD4 T cells respond to alloantigen in a similar manner to wild-type T cells but produce much less  $\gamma$ -interferon and GM-CSF, leading to fewer effector memory T cells in the colon,

decreased levels of inflammatory cytokines, and decreased GVHD pathology and related mortality. Thus, Piper et al place GM-CSF in a key position of bridging innate and adaptive immune responses via recruitment and maturation of antigen-presenting cells in the gut of mice developing GVHD.

The authors also support a broader view of GM-CSF as a key inflammatory cytokine that is involved in a variety of T-cell-mediated pathologies, including neurotoxicity. Fate mapping using fluorescent reporter genes driven by the GM-CSF promoter showed that a subset of CD4<sup>+</sup> T cells expressing interferon- $\gamma$ , tumor necrosis factor, and CXCR6 also expressed GM-CSF and mediated central nervous system inflammation in experimental autoimmune encephalitis.<sup>7</sup> Monoclonal antibodies to the GM-CSF receptor limited the progression and pathology of autoimmune encephalitis in mice,<sup>8</sup> and absence of GM-CSF production by CAR T cells decreased neuroinflammation in murine recipients of CART without compromising their antileukemic activity.<sup>9</sup> Furthermore, the neuroinflammation seen in the murine CART model system can be mitigated by coadministration of a monoclonal antibody to GM-CSF. The current report by Piper et al and emerging data regarding the role of T-cell-mediated production of GM-CSF suggest that therapeutic neutralizing antibodies to GM-CSF (currently

in development) along with small molecule pharmacological inhibitors targeting signaling downstream of Bhlhe40 could reduce or prevent GVHD and neurotoxicity in autoimmune diseases and CART recipients. A number of important questions remain to be explored: What is the ontogeny of these curious CD4<sup>+</sup>CD11c<sup>+</sup> T cells that express a plethora of myeloid genes? Does a homologous population of GM-CSF-secreting T cells exist in humans? Can pathogenic CD4<sup>+</sup>CD11c<sup>+</sup> T cells be selectively depleted? As this field evolves, new therapies for autoimmunity and strategies to enhance anticancer immune responses via GM-CSF in the tumor microenvironment continue to show promise.<sup>10</sup>

*Conflict-of-interest disclosure:* E.K.W. declares receiving research funding and consultancy payments from Partner Therapeutics and Novartis as well as consultancy payments from Humanigen. ■

## REFERENCES

1. Piper C, Zhou V, Komorowski R, et al. Pathogenic Bhlhe40<sup>+</sup> GM-CSF<sup>+</sup> CD4<sup>+</sup> T cells promote indirect alloantigen presentation in the GI tract during GVHD. *Blood*. 2020;135(8):568-581.
2. Partner Therapeutics, Inc. Leukine® sargramostim prescribing information. Available at: <http://www.leukine.com/pi>. Accessed 27 December 2019.
3. Nishinakamura R, Miyajima A, Mee PJ, Tybulewicz VL, Murray R. Hematopoiesis in mice lacking the entire granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 functions. *Blood*. 1996;88(7):2458-2464.
4. Dranoff G, Crawford AD, Sadelain M, et al. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. *Science*. 1994;264(5159):713-716.
5. Markey KA, Banovic T, Kuns RD, et al. Conventional dendritic cells are the critical donor APC presenting alloantigen after experimental bone marrow transplantation. *Blood*. 2009;113(22):5644-5649.
6. Lin CC, Bradstreet TR, Schwarzkopf EA, et al. Bhlhe40 controls cytokine production by T cells and is essential for pathogenicity in autoimmune neuroinflammation. *Nat Commun*. 2014;5(1):3551.
7. Ifergan I, Davidson TS, Kebir H, et al. Targeting the GM-CSF receptor for the treatment of CNS autoimmunity. *J Autoimmun*. 2017;84:1-11.
8. Komuczki J, Tuzlak S, Friebe E, et al. Fate-mapping of GM-CSF expression identifies a discrete subset of inflammation-driving T helper cells regulated by cytokines IL-23 and IL-1 $\beta$ . *Immunity*. 2019;50(5):1289-1304.e6.
9. Sterner RM, Sakemura R, Cox MJ, et al. GM-CSF inhibition reduces cytokine release syndrome and neuroinflammation but enhances CAR-T cell function in xenografts. *Blood*. 2019;133(7):697-709.
10. Hodi FS, Lee S, McDermott DF, et al. Ipilimumab plus sargramostim vs ipilimumab alone for treatment of metastatic melanoma: a randomized clinical trial. *JAMA*. 2014;312(17):1744-1753.

DOI 10.1182/blood.2019004681

© 2020 by The American Society of Hematology