up to one-third of severe hemophilia A patients.

Altogether, it becomes clear why patients report improvements in quality of life and a strong preference to remain on emicizumab.[7] For patients and providers cautious about accepting new advances, the work by Callaghan et al comes as a welcome addition to the previous landmark HAVEN trials. With 2 years of follow-up confirming its safety and efficacy, emicizumab should be considered the standard of care for severe hemophilia A prophylaxis in patients with and without inhibitors. With these exciting data on emicizumab and prospects for other nonfactor therapies around the corner, hemophilia care just keeps getting better.

Conflict-of-interest disclosure: The author receives research funding from and serves on the advisory board for Genentech. ∎

REFERENCES

1. Callaghan MU, Negrier C, Paz-Priel I, et al. Long-term outcomes with emicizumab prophylaxis for hemophilia A with or without FVIII inhibitors from the HAVEN 1-4 studies. *Blood.* 2021;137(16):2231-2242.

2. Oldenburg J, Mahlangu JN, Kim B, et al. Emicizumab prophylaxis in hemophilia A with inhibitors. *N Engl J Med.* 2017;377(9): 809-818.9.

3. Young G, Liesner R, Chang T, et al. A multicenter, open-label phase 3 study of emicizumab prophylaxis in children with hemophilia A with inhibitors. *Blood.* 2019;134(24): 2127-2138.

4. Mahlangu J, Oldenburg J, Paz-Priel I, et al. Emicizumab prophylaxis in patients who have hemophilia A without inhibitors. *N Engl J Med.* 2018;379(9):811-822.

5. Pipe SW, Shima M, Lehle M, et al. Efficacy, safety, and pharmacokinetics of emicizumab prophylaxis given every 4 weeks in people with haemophilia A (HAVEN 4): a multicentre, open-label, non-randomised phase 3 study. *Lancet Haematol.* 2019;6(6):e295-e305.

6. Paz-Priel I, Chang T, Asikanius E, et al. Immunogenicity of emicizumab in people with hemophilia A (PwHA): results from the HAVEN 1-4 studies [abstract]. *Blood.* 2018;132(suppl 1). Abstract 633.

7. Kempton C, Trask P, Parnes A, et al. Development and testing of the Satisfaction Questionnaire with intravenous or subcutaneous hemophilia injection and results from the phase 3 HAVEN 3 study of emicizumab prophylaxis in persons with haemophilia A without FVIII inhibitors. *Haemophilia.* In press.

TRANSPLANTATION

Comment on Song et al, page 2243

Using the binary language of IL-2 to prevent GVHD

Everett H. Meyer | Stanford Medical School

In this issue of *Blood*, Song et al[1] identified a new means of selective blockade of the interleukin 2 (IL-2) pathway during hematopoietic cell transplantation that prevents graft-versus-host disease (GVHD) and augments donor graft-versus-leukemia (GVL) immunity. Using preclinical murine major histocompatibility complex mismatch models of hematopoietic stem cell transplantation, the group used a monoclonal antibody, JES6-1, that binds to IL-2 and selectively interferes with IL-2 binding to specific subunits of the IL-2 receptor (ie, the IL-2 receptor β and γ subunits but not the IL-2 α subunit). This study is among a number of exciting recent developments in our understanding of IL-2 biology and the potential implementation of whole new classes of clinical interventions targeting IL-2.

The importance of the IL-2 pathway in transplantation is underscored by the pivotal role that calcineurin inhibitors played in advancing transplantation. Calcineurin inhibitors act in large part by blunting T-cell receptor signaling and reducing the ability of immune cells to produce IL-2. This includes CD4^{+} helper T cells, which are a major source of IL-2 that drives both their own autocrine expansion and that of other cells such as effector CD8 T cells. Calcineurin inhibitors

such as tacrolimus have known toxicities, but they remain the backbone of most strategies to prevent GVHD.[2] As Song et al again demonstrate in their study, calcineurin inhibitors can impede the GVL effects of donor T cells.

IL-2 has been shown to be plieotropic and can sometimes exert apparently contradictory effects.[3] This is in part because IL-2 binds to dimeric receptors of 2 general types: the IL-2RαIL2Rγ receptor, which is expressed on activated T cells and regulatory T cells (Tregs), and the IL2RβIL-2Rγ receptor, which is expressed on immunologically active T, B, and natural killer (NK) cells. Administration of IL-2 at certain doses can, for example, cause Tregs to expand. This has been used as a clinical strategy to treat chronic GVHD,[4] but at other doses, IL-2 can worsen alloimmune responses and drive T cell or NK reactivity. Monoclonal antibodies that block certain IL-2 receptors or designer IL-2 mutant proteins that bind to the IL-2RαIL-2γ receptors preferentially have been shown to increase Treg numbers and are being evaluated in clinical trials to induce immune tolerance.[5]

Song et al used the antibody JES6-1 to selectively block IL-2 from binding to IL-2 receptors made from the β and common γ chains but not receptors made from the α and common γ chain I (CD25) that Tregs depend on. This reduced donor T-cell proliferation reduced the number of CD4^{+} T cells in the colon and liver, which are GVHD target organs. Inflammatory cytokines such as granulocyte colony-stimulating factor are also reduced. As experimental controls, they used a rat immunoglobulin G (IgG) isotype antibody, and the anti–IL-2 S4B6 antibody that binds to IL-2 allows for some engagement of IL-2 receptors that use the β chain and common γ chain. Compared with control antibodies, Song et al show that JES6-1–treated mice have significantly less severe GVHD and better survival.

In an extensive mechanistic evaluation, the group showed that multiple interrelated pathways are altered to prevent GVHD using this selective antibody approach. Based on other studies, one would first suspect that conventional CD4^{+}CD25^{+}FOXP3^{+} Tregs are primarily responsible for GVHD protection because increasing Treg numbers in other

contexts protects against GVHD.[6] However, Treg percentage did not change in JES6-1–treated recipients. Although Tregs might be necessary for protection (which was not tested), Song et al showed that other pathways are involved. In particular, recipient expression of the immune regulatory receptor PD-L1 is required for GVHD protection because PD-L1^{−/−} recipient mice die from GVHD when treated with JES6-1. Likewise, the group shows that PD-L1 is required for the local colon and liver propagation of CD4^{+} TR1 cells, which make IL-10. CD4^{+} TR1 cells are known to regulate and prevent GVHD.[7] Song et al elegantly shows that IL-10 produced by donor-derived TR1 cells is needed for GVHD protection by the adoptive transfer of IL-10^{−/−} donor cells. They also show increased PD-1:PDL-1–dependent Eomes expression by colon and liver infiltrating donor effector T cells. Higher Eomes expression correlates with anergic and exhausted donor T cells likely incapable of mediating GHVD. One consideration is that Eomes expression in T cells after transplantation can reduce GVL.[8]

In a series of elegant experiments evaluating GVL, Song et al showed that JES6-1 treatment does not prevent immune clearance of the BCL-1 and BC-CML cancer cell lines, whereas treatment with tacrolimus does. Song at al used single cell RNA-Seq and confirmatory flow cytometric of splenic T cells and showed that JES6-1–treated mice have different T-cell immune reconstitution of CD4 and CD8 populations in the spleen that do not appear anergic vs what is seen in GVHD target tissue. This may promote GVL. Song et al present some evidence that NK cells are not responsible for GVL, but further mechanistic studies are needed to understand the preservation of GVL in this model, including evaluating alternative hypotheses such as the increase in TR1 cells that may be responsible for increased GVL.

Overall, the study by Song et al reinforces the concept that IL-2 and donor CD4^{+} T cells in the local tissue microenvironment play a major role in GVHD pathophysiology through the PD-L1 pathway and the complex interplay of cell populations such as Treg, TR1, and effector T-cell subsets. The authors also make the fundamental observation that targeting the IL-2 pathway more precisely may promote GVL immunity.

REFERENCES

1. Song Q, Wang X, Wu X, et al. Tolerogenic anti–IL-2 mAb prevents graft-versus-host disease while preserving strong graft-versus-leukemia activity. *Blood*. 2021;137(16):2243-2255.

2. Martinez-Cibrian N, Zeiser R, Perez-Simon JA. Graft-versus-host disease prophylaxis: Pathophysiology-based review on current approaches and future directions. *Blood Rev.* 2020;100792:100792.

3. Abbas A, Trotta E, Simeonov D, et al. Revisiting IL-2: biology and therapeutic prospects. *Sci Immunol*. 2018;3(25):eaat182.

4. Koreth J, Matsuoka K, Kim HT, et al. Interleukin-2 and regulatory T cells in graft-versus-host disease. *N Engl J Med*. 2011;365(22):2055-2066.

5. Mizui M. Natural and modified IL-2 for the treatment of cancer and autoimmune diseases. *Clin Immunol*. 2019;206:63-70.

6. Blazar BR, MacDonald KPA, Hill GR. Immune regulatory cell infusion for graft-versus-host disease prevention and therapy. *Blood*. 2018; 131(24):2651-2660.

7. Roncarolo MG, Gregori S, Bacchetta R, Battaglia M. Tr1 cells and the counter-regulation of immunity: natural mechanisms and therapeutic applications. *Curr Top Microbiol Immunol*. 2014;380:39-68.

8. Zhou M, Sacirbegovic F, Zhao K, Rosenberger S, Shlomchik WD. T cell exhaustion and a failure in antigen presentation drive resistance to the graft-versus-leukemia effect. *Nat Commun*. 2020;11(1):4227.

VASCULAR BIOLOGY

Comment on Zirka et al, page 2256

VT duets: inflammation/coagulation—wall/flow

Jose Antonio Diaz | Vanderbilt University Medical Center

In this issue of *Blood*, Zirka et al[1] investigated the role of Slc44a2 in thrombosis. Specifically, they focused on determining how Slc44a2 expressing either the human neutrophil antigen (HNA)-3a or HNA-3b on neutrophils could modulate their adhesion and activation of von Willebrand factor (VWF) under certain flow conditions. Prior genome-wide association studies linked expression of HNA-3b on the Slc44a2 protein with a decreased risk of venous thrombosis (VT) in humans. Slc44a2 is a ubiquitous transmembrane protein and a receptor for VWF. The authors examined how neutrophil Slc44a2 expressing either HNA-3a or HNA-3b modulated adhesion and activation on VWF under flow. Transfected HEK293T cells or neutrophils homozygous for the HNA-3a– or the HNA-3b–coding allele were perfused in flow chambers coated with VWF, mimicking venous shear rates. HNA-3a expression was required for Slc44a2-mediated neutrophil adhesion to VWF at those shear rates. Adhesion was enhanced when neutrophils were activated with lipopolysaccharide. Specific shear conditions with high neutrophil concentrations worked like a "second hit," inducing the formation of neutrophil extracellular traps. Neutrophil mobilization was also measured by intravital microscopy in venules. Mice lacking Slc44a2 showed a massive reduction in neutrophil recruitment in inflamed mesenteric venules.

They concluded that Slc44a2/HNA-3a is important for the adhesion and activation of neutrophils in veins during inflammation and when submitted to specific shears similar to those in veins. The authors included blood flow as part of their methodology, which is a critical component in the thrombosis process in blood vessels. Although the flow devices available have limitations, these types of tools bring blood flow into the equation. Blood flow could be even more critical