traveling through the bloodstream. There may be methods to improve the half-life of the anti-β3 scFv in the bloodstream and this would be obvious improvement now that the exciting basic proof-of-principle data exist.

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

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Comment on Brennan et al, page 2899

α-1 antitrysin DAMPens GVHD

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In this issue of *Blood*, Brennan et al report that a noninfectious damage-associated molecular pattern (DAMP), heparan sulfate (HS),[1] aggravates graft-versus-host disease (GVHD) and that this enhanced severity can be dampened by administration of serine protease inhibitor α-1 antitrysin (AAT).[2]

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective therapy against many hematologic diseases. However, despite the curative potential its application has been limited by its many complications, most notably GVHD. Detection of tissue damage by innate immune cells using pattern-recognition receptors (PRRs) that sense noninfectious molecular signatures called DAMPs is known to augment adaptive immunity.[3] After conditioning for allo-HSCT, both infectious and damage-associated noninfectious signals (DAMPs) enhance severity of GVHD.[4] However, the nature and type of DAMPs implicated in augmenting GVHD remain poorly understood. Brennan and colleagues provide an important piece of the puzzle by demonstrating that HS, an extracellular matrix component, enhanced host antigen-presenting cell function in a TLR4-MyD88–dependent manner and accentuated alloreactive donor T cell–mediated GVHD.[2]

Excessive inflammation underlies pathologic immunologic processes, including GVHD.[5] Although the GVHD-enhancing aspects proinflammatory cytokines is well appreciated, much remains unknown about the specific instigators that induce, perpetuate, and accentuate inflammation after allogeneic HSCT.[4] The classic instigators of inflammation are the microbial-derived pathogen-associated molecular pattern molecules (PAMPs) and the nonmicrobial tissue damage-associated DAMPs that are recognized by PRR-bearing immune cells.[3] Several endogenous molecules such as high mobility group1, adenosine-5′-triphosphate, heat shock protein (HSP) 70, fibronectin, HS, hyaluronic acid, and uric acid can function as DAMPs. Many PRRs, including the TLR family, recognize both PAMP and DAMP motifs.[3] Activation of innate immunity by these motifs shapes adaptive immunity.[3] Experimental studies have only recently begun to uncover the role of specific DAMPs (likely generated in the allo-HSCT hosts from either disease or conditioning or GVHD-related tissue damage) in modulating GVHD severity.[6]

Brennan et al analyzed in vitro impact of several DAMPs in enhancing alloreactive T-cell proliferation.[2] They found that only HS and HSP70 significantly promoted alloreactive T-cell expansion. This in vitro enhancement of alloreactive T-cell proliferation by HS was dependent on the expression of TLR4 and MyD88 in the stimulators (dendritic cells [DCs]) and not in the responders (allo-T cells). HS also promoted in vitro maturation and secretion of proinflammatory cytokines from DCs in a predominantly TLR4-MyD88–dependent manner. Using transfection studies of epithelial cell lines they found that HS caused NF-κB translocation in a TLR4-dependent manner. They explored the relevance of HS-mediated inflammation, in vivo, by measuring HS levels after MHC matched, minor antigen mismatched experimental allogeneic bone marrow transplantation (BMT). HS levels increased a week after BMT and returned to baseline by 3 weeks after BMT in the allogeneic recipients. The kinetics suggest that the increase in this extracellular matrix moiety, HS, might be more a consequence of GVH-induced damage than perhaps from conditioning-related tissue damage. Using 4C-TCR-trangenic T cells in a creative experimental model system that employed an MCH mismatched BMT model, they suggest persistence of host class II–expressing antigen presenting cells (APCs) at the time of peak levels of HS. However, it is unclear whether the kinetics of HS levels in sera are the same in MHC matched and mismatched BMT systems. Furthermore, the data do not directly show whether the class II–expressing cells are indeed radio-resistant host hematopoietic APCs (as suggested) and/or if they are also nonhematopoietic APCs. It is also formally possible that PAMPs like lipopolysaccharides that are recognized by TLR4 may also simultaneously contribute in an additive or synergistic manner to the role of HS in aggravating GVHD. Nonetheless, administration of an HS mimetic further accelerated GVHD, indicating an important role for HS in aggravating GVHD. In addition, HS levels were elevated in the sera of patients from a relatively small and mixed cohort of allogeneic BMT recipients and correlated temporally with onset of GVHD in these patients. In light of the levels of HS being higher in patients with Hunter syndrome (mucopolysaccharidosis Type II), it would be worth exploring whether these patients suffer from higher-than-anticipated rates of GVHD after allogeneic BMT. Importantly and consistent with previous observations, in multiple experimental models when allogeneic recipients were treated with AAT, a serine protease inhibitor that is clinically available and known to inhibit elastase, GVHD was significantly attenuated.[7-9] AAT-induced reduction in GVHD correlated with reduced HS levels and was observed only in wild-type but not in TLR4-deficient mice. Brennan and colleagues posit that AAT reduced GVHD

through inhibition of elastase-induced cleavage and release of HS from the extracellular matrix in host tissues.

These observations have significant implications for our understanding of the biology of GVHD and also toward potential development of novel therapeutic strategies. They add to the growing body of evidence demonstrating the impact of DAMPs in regulating alloreactivity and indicate that targeting specific DAMPs might regulate GVHD.[6] This work echoes the recent observations on the anti-inflammatory effects of AAT.[7-9] The study suggests, but does not directly demonstrate, that AAT "dampens" GVHD through the reduction of the DAMP, HS. While clarifying, like all interesting observations, the study raises additional questions. The critical role for TLR4-MyD88 pathways in aggravating GVHD is in contrast to recent findings by Li et al.[10] This could represent a potential strain dependency of the observations made by Brennan et al. It should remind us that insights from animal models, especially when they conflict, must be extrapolated to humans with caution. The increased levels of HS observed at the onset of GVHD in humans nonetheless add depth to Brennan and colleagues' observations. These observations will ideally have to be confirmed prospectively in a larger and more uniform cohort of patients. However, intriguingly, AAT mitigated GVHD in multiple models. Along with previous observations, this study further underscores the potent effects of AAT in modulating inflammation and immunity.[7-9] Brennan et al's observations and those by another study[8] suggest that in some GVHD models, the immunologic effects of AAT might be directly mediated by its antiprotease activity. However, this notion remains to be tested directly and definitively in these models. Indeed, whether the basic tenet that all functions of AAT are directly attributable to its ability to target elasatse remains to be rigorously tested.[9] The key cellular targets and the critical molecular mechanisms of AAT-mediated immune regulation are thus unknown. Nonetheless, in light of the clinical availability of AAT and its long track record of safety in humans, the observations of Brennan and colleagues along with those of others[7-9] suggest that administration of AAT may be considered as an adjunct to standard therapy, in carefully designed clinical trials to mitigate GVHD.

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

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Comment on Horan et al, page 2918

HLA factors in transplantation for nonmalignant hematologic disorders

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In this issue of *Blood*, a study by Horan and colleagues shows that differences in the HLA alleles of patients and unrelated donors in hematopoietic stem cell transplantation (HSCT) for nonmalignant diseases result in increased risk for adverse treatment outcome.[1] This is the largest dataset examined so far for the evaluation of HLA mismatches in HSCT for nonmalignant diseases. It includes predominantly pediatric patients diagnosed with 39 diseases. Many patients received nonmyeloablative conditioning; a significant proportion of the infused grafts were depleted of T-lymphocytes; 6 diseases account for 77% of the cases.

Currently, the criteria used for selection of unrelated donors for nonmalignant diseases derive from studies performed in HSCT for patients with malignant diseases[2]; in the latter it was found that the HLA mismatches associated with patient mortality. The study by Horan et al shows that HLA mismatches in HLA-A, -B, -C, and DRB1 loci also have a significant impact in outcome of HSCT for nonmalignant diseases. This study provides useful insights that can be applied to the definition of unrelated donor selection criteria for nonmalignant diseases. The nonmalignant disease cohort[1] differs significantly from those examining HSCT for hematologic malignancies[2] in age, conditioning regimens, and graft composition. In the nonmalignant disease study the incidence of graft failure was at least 2 to 3 times higher than in the cohorts of malignant diseases. In nonmalignant disease transplantation, many patient deaths (29.8%) were associated with graft failure. The multivariate analyses showed that the occurrence of a single or a double mismatch in HLA-A, -B, -C, or DRB1 loci associated with graft failure; 2 HLA mismatches were also associated with mortality. The single HLA mismatch associated with patient death only in the univariate analysis. Interestingly, in nonmalignant disease HSCT, the mismatched HLA loci did not associate with any type or grade of graft-versus-host disease (GVHD). Horan and colleagues noted that the absence of an association between HLA mismatch and acute GVHD was likely because most of the patients received a lymphocyte-depleting antibody and/or received an ex vivo T cell–depleted graft. These findings contrast with those made in HSCT for malignant diseases; in the latter, the HLA mismatches associate risks for acute