center large study provides implied uniformity of supportive care as a strength. However, it is important to note that these conclusions must be restricted to patients with grade I-II acute GVHD, as less than 3% of patients treated with low-dose prednisone had severe (grades III-IV) acute GVHD. In addition, this analysis was restricted to adults. Although age has not been a consistent factor in the response to acute GVHD therapy, future trials should include children.

Inherent in the retrospective design of this study is the presence of potentially confounding variables. The starting steroid dose was chosen at the discretion of the attending physician and as the authors discuss, there must certainly have been a bias toward giving the higher dose steroids to patients with more fulminant GVHD. This is suggested by the fact that only 2.6% of the low-dose steroid patients had severe GVHD compared with 16.3% patients in the standard-dose steroid group.

The 2 treatment groups also differed in a number of important clinical variables. A higher proportion of the low-dose patients had more frequent gut and only limited or no skin GVHD compared with the standard-dose group. The high incidence of gut GVHD in low-dose steroid recipients led to greater use of oral beclomethasone dipropionate, an agent that has been shown to incur a steroid sparing effect and survival advantage in an earlier study.[5]

Notably, despite being associated with less toxicity, the use of low-dose steroids was not associated with less transplantation-related mortality or overall mortality. Although relapse did not differ between all patients in the 2 steroid groups, in a subgroup analysis, risk of relapse was 1.5-fold higher in low-dose steroid recipients than in standard-dose recipients. The groups were balanced with respect to disease risk; however, future prospective trials are needed to determine the impact of steroid dose on this outcome.

In the race to improve outcomes in the treatment of acute GVHD, Mielcarek et al provide compelling evidence that, at least for adult patients with grades I-II acute GVHD, the start line may have been moved back to 1 mg/kg per day. Future prospective studies are warranted to determine the optimal starting dose and to carefully evaluate its consequences for both adults and children and especially for those with severe GVHD. In addition, further identification of patient and graft characteristics predictive of response will lead to a more tailored approach to acute GVHD therapy in the future.

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Comment on van den Biggelaar et al, page 3102

VWF and FVIII: the origins of a great friendship

Sandra L. Haberichter MEDICAL COLLEGE OF WISCONSIN

In this issue of *Blood*, van den Biggelaar and colleagues demonstrate the cotargeting of FVIII and VWF to secretory granules in the absence of a high-affinity interactions between VWF and FVIII, using recombinant VWF type 2N variants.

In addition to mediating the adhesion of platelets to subendothelial tissue at the site of vascular injury, von Willebrand factor (VWF) also serves as the carrier protein for coagulation factor VIII (FVIII) in plasma. The initial point of physical association between these 2 proteins remains unclear. When desmopressin (DDAVP) is administered to mild hemophilia or von Willebrand disease (VWD) patients, both FVIII and VWF are released into plasma. Presumably, VWF is released from endothelial cell Weibel-Palade bodies. While the liver is the major site of FVIII synthesis, the cell within the liver producing FVIII has not been definitively identified. Recent publications suggest that FVIII may be present in selected endothelial populations including human lung microvascular endothelial cells and murine sinusoidal endothelial cells.[1,2]

Several studies have demonstrated that expression of FVIII in VWF-producing cells, such as HUVEC, cultured megakaryocytes, and platelets, results in colocalized storage of VWF and FVIII in endothelial cell Weibel-Palade bodies or in α-granules of platelets and megakaryocytes.[3-6] These studies have suggested that FVIII-regulated storage is secondary to VWF storage and results from a high-affinity VWF-FVIII association early in the secretory pathway. Seemingly in contrast to this conclusion is the observed concomitant release of both VWF and FVIII in type 2N VWD patients after DDAVP administration.[7] Type 2N VWD variants are characterized by a markedly decreased binding affinity for FVIII caused by homozygous or compound heterozygous mutations in VWF that impair FVIII binding. In these patients, the loss of high-affinity VWF-FVIII binding does not promote stabilization of FVIII in plasma and FVIII is degraded fairly rapidly. Given the lack of FVIII binding to 2N variants of VWF, the cellular source of the DDAVP-releasable pool of FVIII is an open question.

The current study by van den Biggelaar et al, which uses several recombinant type 2N VWF variants, provides valuable insight into the relationship between assembly of the VWF/FVIII complex and the cotrafficking of VWF and FVIII to the regulated secretory pathway.[8] The authors employ complementary techniques, Surface Plasmon Resonance (SPR) and a pseudo-equilibrium binding assay to demonstrate a range of mildly to severely reduced FVIII-binding affinity of the 2N variants. Using an HEK293 cell expression system, the authors elegantly demonstrate that, despite the FVIII binding defects, all type 2N variants were able to target coexpressed FVIII and P-selectin to the VWF-containing pseudo–Weibel-Palade bodies. This finally provides a mechanistic basis for the observed DDAVP-induced release of FVIII and VWF in type 2N VWD patients.

The data presented in this study indicate that assembly of a high-affinity VWF/FVIII complex is not required for cotrafficking of the 2 proteins, but rather FVIII storage may be secondary to the VWF-dependent biogenesis of the secretory granule itself. In a study by Yarovoi et al, expression of FVIII in VWF-deficient platelets resulted in some storage of FVIII in α-granules.[9] This provided further evidence that interaction with VWF is not absolutely necessary for regulated storage of FVIII. Other yet unidentified cell-specific factors are likely to play a role in granule formation and protein targeting. An additional noteworthy observation from this study is the change in granule morphology from elongated, rodlike granules to round vesicles when FVIII is present in the granule. This type of morphologic change has been observed in previous studies examining other VWF variants and, in sum, these studies suggest that granule morphology may depend on the intragranule content. As pointed out by the authors, endothelial cells have organ-specific characteristics, and variations in expression and morphology may be dependent on the particular lineage of the vascular tree.

This study by van den Biggelaar et al provides a molecular explanation for the concomitant release of FVIII and VWF in type 2N VWD patients and demonstrates that a high-affinity VWF/FVIII complex is not necessary for targeting FVIII to the regulated secretory pathway. This study provides an answer to the long-standing question of FVIII release in type 2N patients and also creates new questions regarding FVIII biosynthesis and granule biogenesis.

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