	Heparin preparation	Severity of trauma		*P* value
		Major	Minor	
A. Anti-PF4/heparin antibody seroconversion				
	UFH	17/100 (17.0%)	4/216 (1.9%)	<0.0001
	LMWH	5/124 (4.0%)	0/174 (0%)	0.012
	P	0.0014	0.132	
B. Clinical HIT				
	UFH	4/100 (4.0%)	0/216 (0%)	0.010
	LMWH	1/124 (0.8%)	0/174 (0%)	0.416
	P	0.175	1.0	

Anti-PF4/heparin antibodies were measured using a combination of enzyme-immunoassays, and thus indicate antibodies of either IgG, IgA, and/or IgM classes.
* Minor trauma also includes patients who did not undergo surgery.
Abbr.: HIT, heparin-induced thrombocytopenia; LMWH, low-molecular-weight heparin (certoparin); UFH, unfractionated heparin

Anti-PF4/heparin immunization frequency and HIT frequency in relation to type of heparin (UFH vs LMWH) and severity of trauma (major vs minor*).

The data clearly indicate that both heparin type and trauma severity are strong and independent predictors of the anti-PF4/heparin immune response. Consequently, the highest risk of immunization (17%) and of clinical HIT (4%) was seen in patients who received UFH thromboprophylaxis after major trauma. At the other end of the spectrum, not even a single immunization event occurred in 174 patients who received LMWH thromboprophylaxis after minor trauma.

A quick survey of the data in the table suggests that the effect of trauma severity was at least as great as that due to heparin type. Most notably, the frequency of anti-PF4/heparin immunization was approximately twice as high in patients who received LMWH after major trauma (5 of 124, 4.0%) compared with those who received UFH after minor trauma (4 of 216, 1.9%). Indeed, when the Greifswald group applied logistic regression analysis, adjusting for confounders such as age, sex, and type of heparin, the odds ratio for developing any immune response in major trauma compared with minor trauma was 7.98 (95% CI, 2.06-31.00; $P = .003$). This mirrors the approximately 10-fold greater frequency of HIT that has been shown with UFH compared with LMWH.[2,3]

The authors speculate that increased release from platelets of PF4 in the context of major surgery, or perhaps a greater degree of inflammation—a plausible potentiator of the anti-PF4/heparin immune response—might be responsible. Perhaps a "double HIT"—simultaneous antigen exposure plus a proinflammatory "danger signal"—is needed for triggering a strong immune response. To date, this concept of proinflammatory potentiators has been largely suggested in case reports. For example, acquired hemophilia has been observed anecdotally to occur in the context of infection, surgery, and malignancy.[4-6] More powerfully, this study by Lubenow et al demonstrates through compelling clinical trial data that a nondrug factor—severity of trauma—is of major importance in potentiating the heparin-induced immune response.

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● ● ● TRANSFUSION MEDICINE

Comment on Canault et al, page 1835

p38: signaling improved platelet storage?

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During storage, platelets become gradually impaired in activation and signaling responses. In this issue of *Blood*, Canault and colleagues demonstrate that storage-induced shedding of platelet receptors GP1bα and GPV is mediated by p38 MAP kinase and inhibition of this pathway improves the function and posttransfusion recovery of stored platelets.[1]

Therapeutically, platelet concentrates are crucial for transfusion into thrombocytopenic patients, particularly when bleeding. However, the efficacy of these transfusions is limited by the shelf life of the platelet concentrates and their diminishing function over time. Therefore, it is crucial to understand how to optimally store platelets to preserve their hemostatic function once transfused. In addition, it is important to maintain the suppression of platelet markers that could contribute to their clearance after transfusion.

Platelet storage lesion is a term that describes both the biochemical and structural changes that occur in platelets during storage. Morphologic and functional alterations have been characterized in stored platelets and include shape change, reduction in activation by agonists, secretion of platelet granules, blebbing, and exposure of surface phosphatidylserines. Less is known about the biochemical properties regulating these changes. Recently, inhibition of PI3-kinase–dependent Rap1 activation has been reported to reduce both $\alpha_{IIb}\beta_3$ activation and α-granule release, improving platelet survival during storage.[2]

During storage, platelets shed adhesive surface glycoproteins. In this issue of *Blood*, Canault et al investigate another signaling pathway responsible for platelet receptor shedding that is known to alter platelet function.[1] They examine the role of p38 MAP kinase in GPIbα and GPV shedding from the

platelet surface through tumor necrosis factor-α–converting enzyme (TACE/ADAM17) and how this affects stored platelet function and survival. Using mice that express a nonfunctional form of TACE, they confirm that TACE is responsible for the cleavage of GP1bα and GPV, as previously reported.[3-5] Canault et al show that inhibition of TACE is important in posttransfusion clearance of platelets.[1] Shedding of both glycoproteins by TACE is not mediated through PKC, MEK/ERK, or caspases, as demonstrated by pharmacologic inhibitor work.[1] Instead, inhibition of p38 MAPK significantly reduces the shedding of GPIbα and GPV from the platelet surface.[1] Inhibition of p38 MAPK does not affect platelet function but does affect increased platelet recovery and improved platelet function after transfusion.[1]

The work presented by Canault et al[1] suggests that treating all stored platelets with a p38 MAPK inhibitor would increase the efficacy of platelet transfusions. However, questions arise over whether or not this is possible in humans. Posttransfusional function of platelets and survival of transfused mice suggest that the addition of a p38 MAPK inhibitor will not have any adverse effects. Importantly, this is inconsistent with previous data using human platelets in which inhibition of p38 MAPK resulted in the loss of platelet aggregation induced by collagen[6,7] or by low-dose thrombin,[7] although some recovery occurred at higher concentrations of the agonists.[6,7] Is it possible that the differences can be attributed to the model being studied, that is, human platelets versus mouse platelets? Clearly, further experimentation is needed to clarify the clinical effects of long-term storage with a p38 MAPK inhibitor on platelet function, survival, and patient-specific platelet reactivity.

Another issue with this approach is that inhibition of MAP kinase as a means of preventing platelet storage disease is nonspecific. It is well established that this pathway regulates many parts of platelet activation (not just shedding) and many functions in the vasculature. Animal studies looking at the inhibition p38 MAPK in high-salt, high-fat diets have shown a reduction in blood pressure, and improved endothelial-dependent and -independent vasorelaxation.[8] Hypoxia-induced endothelial dysfunction can be reversed with inhibition of p38 MAPK by improving vasorelaxation, increasing NO production, and reducing superoxide levels.[9] Although these properties, that is, vasodilation, may be beneficial in the setting of elevated blood pressure, vessel dilation can be dangerous in the setting of hemorrhage. Clearly, the global effect of p38 MAP kinase inhibition on the vasculature relevant to bleeding would need to be examined.

Can MAP kinase inhibition be used to maintain the integrity and functionality of platelets stored for transfusion? Whereas this article presents some exciting mechanistic data, more questions are raised concerning the clinical relevance of this approach. As there are many other factors that can affect platelet function during storage, such as bacterial contamination and activation by plasma products, that are independent of p38 MAPK signaling, these interesting data stress the importance of taking a broad view of the problems involved with platelet transfusion.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● TRANSPLANTATION

Comment on Cai et al, page 1669

Separation of GVHD and GVL

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CD4$^+$Foxp3$^+$ T_{regs} suppress antitumor responses using a granzyme B–dependent mechanism while the regulatory control of GVHD by T_{regs} exploits a distinct mechanism that is granzyme B–independent.

Allogeneic bone marrow transplantation (BMT) is a viable therapeutic option for the treatment of a variety of hematologic malignancies. A major complication of allogeneic BMT is graft-versus-host disease (GVHD), in which the alloreactive T cells transferred along with the bone marrow graft respond to antigenic differences expressed on host tissues. Although this posttransplantation complication is a significant cause of morbidity and mortality after allogeneic BMT, GVHD does appear to have a significant antitumor benefit, often termed a graft-versus-leukemia (GVL) effect. Relapse rates in patients who develop GVHD are considerably lower compared with rates in patients who do not develop this complication after transplantation. Over the past several decades, attempts to identify and separate specific immune effector mechanisms that mediate GVHD and GVL have been largely unsuccessful. Sometimes the best way to make progress in a field is not to keep trying to get further down the same road, but to take the road less traveled. In this issue of *Blood*, Cai and colleagues have uncovered the fact that suppression of GVHD and GVL appear to function by different mechanisms.[1] If these differences in the regulation of GVHD and GVL effector mechanisms can be exploited, the potential for therapeutic enhancement of allogeneic