blood advances

TO THE EDITOR:

Is GPIbα really a master regulator of platelet activation?

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We read with great interest the recent publication from Yan et al in *Blood Advances* entitled "Essential role of glycoprotein Ibα in platelet activation."[1] The study presents findings showing that the deletion of 10 amino acids (aa) from the cytoplasmic tail of glycoprotein Ibα (GPIbα) in mouse platelets results in defective platelet responses downstream of the von Willebrand factor (VWF)–GPIbα interaction but also all other major platelet receptors including GPVI, thromboxane receptor, P2Y12, and protease-activated receptor 4. Unsurprisingly, these mice exhibited impaired hemostasis and reduced thrombus formation after laser- or $FeCl_3$-induced endothelial injury of arterioles and were protected in a model of pulmonary thromboembolism.[1] Mechanistically, this thromboprotective phenotype was linked to reduced protein kinase C (PKC) activity in $10aa^{-/-}$ platelets. Complementary to these findings, the authors also show that a myristoylated peptide of GPIbα (MPαC),[2] previously shown to prevent the binding of 14-3-3ζ to GPIbα, is able to activate platelets via PKC in washed platelets but not in platelet-rich plasma. Although the authors should be commended for the amount of data generated in this article, there are several crucial issues with the study that requires further consideration.

Primarily, we would like to comment on the validity of the $10aa^{-/-}$ mouse model. Due to the difficulty of genetically modifying platelets and the limited availability of specific inhibitors of platelet receptors, the creation of novel mouse models is invaluable to provide insights into molecular mechanisms of platelet signaling/activation. There are many GPIbα mouse models nicely reviewed by Jerry Ware,[3] and particularly relevant in the context of the present $10aa^{-/-}$ model are the hTg^{Y605X} and $Gp1b\alpha^{\Delta sig/\Delta sig}$ mice, which harbor a deletion of the last 6 or 24 aa of the intracellular tail of GPIbα, respectively.[4,5] The hemostatic responses in the $10aa^{-/-}$ mice are quite different from the ones observed in hTg^{Y605X} and $Gp1b\alpha^{\Delta sig/\Delta sig}$ mice.[4,5] Briefly, none of these 2 mice exhibited altered hemostasis. The increase in bleeding time observed in $10aa^{-/-}$ mice could perhaps have been influenced by a more severe challenge to the mouse hemostatic system (5-mm tail transections vs 2 mm for $Gp1b\alpha^{\Delta sig/\Delta sig}$ mice). Collagen related peptide- as well as thrombin- and adenosine 5′-diphosphate (ADP)–induced platelet aggregation was normal in $Gp1b\alpha^{\Delta sig/\Delta sig}$ mice as well as platelet accumulation and fibrin deposition after laser-induced thrombus formation.[5] However, a significant decrease in P-selectin and activated $\alpha_{IIb}\beta_3$ expression was observed in collagen related peptide-stimulated $Gp1b\alpha^{\Delta sig/\Delta sig}$ compared with wild-type platelets but not when platelets were stimulated with ADP or thrombin.[5] In light of the results from Yan et al showing a thromboprotective phenotype for $10aa^{-/-}$ mice in laser- or $FeCl_3$-induced thrombosis models of microvessels, it is also important to highlight that, in a $FeCl_3$-induced carotid thrombosis model, hTg^{Y605X} mice were unable to form stable thrombi and exhibited increased embolization events, despite normal hemostasis and platelet counts.[6] It is unclear whether this defect in hTg^{Y605X} mice can be attributed to decreased signaling or lower affinity of human GPIbα for murine VWF.[7] Although Yan et al do mention the differences observed in their mouse model compared with hTg^{Y605X} and $Gp1b\alpha^{\Delta sig/\Delta sig}$ mice, they have not considered why this might be.

There is very little information on the generation of $10aa^{-/-}$ mice via clustered regularly interspaced short palindromic repeats-associated protein 9 technology. Data provided verifying that only the last 10 aa of the cytoplasmic tail of GPIbα have been deleted with no other changes are not entirely convincing.

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This now becomes important for interpreting the discrepancies between different platelet response phenotypes among different mouse models. The western blot using an anti-GPIbα C-terminal antibody (Figure 4B from Yan et al[1]) suggests that indeed a portion of the tail of GPIbα has been deleted; however, it is also important to highlight no further information on this antibody is provided. Moreover, the loss of binding does not confirm the precise deletion of the last 10 aa of GPIbα. It is, therefore, essential that the GPIbα genomic DNA flanking the site of modification is sequenced to ensure that the desired deletion and stop codon have been correctly generated and at the right genomic location. Omitting such details limits the readers' ability to establish how reliable the presented data are and its significance, given that already generated models gave very different phenotypes.

Results using the MPαC peptide also seem unclear. MPαC is a GPIbα C-terminal sequence peptide with phosphorylated Ser^{609} and has been shown to inhibit VWF-induced platelet aggregation in platelet-rich plasma.[2] They also have previously shown that it diminished platelet aggregation triggered by low-dose thrombin in washed human platelets at 10-μM concentration.[8] Although they have reproduced these results (see supplemental Figures 2 and 9 from Yan et al[1]), the author's in vitro results demonstrate that 100-μM MPαC induces activation of washed human and mouse platelets but not when platelets are in plasma. Based on this, it is therefore difficult to reconcile how MPαC could exert an effect in vivo by rescuing the defects seen in $10aa^{-/-}$ washed platelets. The authors did not discuss the possible off-target impacts of MPαC, nor do they make consideration of the mechanism of entry for MPαC into platelets in plasma-free vs whole-blood conditions. To add to the complexity of the MPαC mode of action, the authors have previously demonstrated that, when injected into C57BL/6J mice, this peptide delayed occlusion time after $FeCl_3$ injury of the carotid,[9] which is in stark contradiction with the present findings in vivo in which it shortens the occlusion time in $10aa^{-/-}$ mice while having no effect on wild-type animals.

Finally, the authors primarily focus on the role the GPIbα cytoplasmic tail has on moderating PKC activity via sequestering 14-3-3 isoforms, a concept they proposed 19 years ago, and conclude this is how the GPIbα cytoplasmic tail regulates platelet activation.[2] Here again, it is puzzling that the authors have not even considered there could be additional mechanisms taking place; particularly because the GPIbα cytoplasmic tail has other binding partners it uses to transduce platelet signals, which the authors have not discussed. It is very difficult to understand how this 14-3-3 sequestration model works in different scenarios: normal and shorter GPIbα in the presence of the MPαC peptide, with or without platelet agonists.

In summary, Yan et al assign an important role to the tail of GPIbα in platelet signaling and activation through its binding to 14-3-3, promoting PKC activation. Although this is an elegant concept, we suggest that these conclusions deserve additional thoughts based on the lack of key information related to the tools and models used and especially considering that patients with Bernard Soulier syndrome generally have normal platelet aggregation profiles with agonists other than ristocetin.[10,11] Few case studies, nevertheless, report defective platelet aggregation with ADP or collagen, but here again, the difficulty of adequately comparing the aggregation profiles of platelets from patients with Bernard Soulier syndrome with healthy platelets due to their size and low counts should be acknowledged and perhaps deserve further investigation.

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