At the MERcy of platelet primers

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COMMENTARY

At the MERcy of platelet primers

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See also Branchford BR, Stalker TJ, Law L, Acevedo G, Sather S, Brzezinski C, Wilson KM, Minson K, Lee-Sherick AB, Davizon-Castillo P, Ng C, Zhang W, Neeves KB, Lentz SR, Wang X, Frye SV, Shelton Earp H III, DeRyckere D, Brass LF, Graham DK, Di Paola JA. The small-molecule MERTK inhibitor UNC2025 decreases platelet activation and prevents thrombosis. This issue, pp 352–63.

In this issue of the *Journal of Thrombosis and Haemostasis*, Branchford *et al*. [1] present novel data on the role of Mer tyrosine kinase in platelet function and thrombosis. Mer forms, together with Axl and Tyro3, the TAM receptor family of growth arrest-specific (Gas) receptors. The vitamin K-dependent protein Gas6 is regarded as a platelet primer, like other molecules such as epinephrine, insulin-like growth factor-1, insulin-like growth factor-2, matrix metalloproteinase-1, matrix metalloproteinase-2, oxided LDL, prostaglandin E2, soluble CD40 ligand, and thrombopoietin [2]. Platelet primers are platelet agonists that are unable to stimulate platelets by themselves, but potentiate activation processes evoked by other agonists. Gas6 is released by a number of cell types, including endothelial cells, vascular smooth muscle cells, bone marrow, and, to a limited extent, human platelets (reviewed in [3]). Binding of Gas6 to its receptors is thought to result in dimerization of two receptor monomers, which provokes autophosphorylation of tyrosines in the cytosolic moieties of the receptor. Intracellular signaling via phosphatidylinositol 3-kinase (PI3K) isoforms plays a central role in mediating the effects of Gas6 and other platelet primers, with different PI3K isoforms having divergent roles. Gas6 levels are elevated in systemic inflammatory disorders, such as sepsis and lupus erythematosus, and in venous thromboembolic disease (reviewed in [3]). Healthy subjects with elevated Gas6 levels have been found to have an approximately 10-fold higher risk of being aspirin-pseudoresistant, i.e. subject to persistent platelet aggregation despite inhibition of thromboxane A2 production by aspirin [4]. The latter suggests that priming of platelets might be a mechanism of resistance to antiplatelet therapy.

It is a topic of debate whether only Mer is present on the surfaces of human and mouse platelets [5,6], or whether Axl and Tyro3 are also present [7–10]. It is conceivable that Axl and Tyro3 are expressed at such low levels that they remain below the detection limit of some of the assays used. Unfortunately, even highly sensitive platelet proteomics studies have failed to shed light on this matter, because, in contrast to what has been found in other cell types [11], neither the TAM receptors nor Gas6 could be detected in human or mouse platelets [12,13]. Interestingly, however, knocking out *Mer*, *Axl* or *Tyro3* in mice yields a moderate antithrombotic effect [8]. TAM receptors also modulate inflammation and cell growth, and, importantly, appear to have distinct roles in these processes. $Mer^{-/-}$ mice show several autoimmune-like features, whereas $Tyro3^{-/-}$ mice develop neurologic defects, and $Axl^{-/-}$ mice have vascular defects similar to those seen in $Gas6^{-/-}$ mice (reviewed in [3]). In addition, Axl is predominantly overexpressed in a variety of human cancers [14].

How important is Mer in mediating thrombosis and hemostasis? Branchford *et al*. [1] thoroughly addressed this question by investigating the effects of a small-molecule inhibitor of Mer, UNC2025, on platelet function and thrombosis. It should be noted that UNC2025 is equally potent in inhibiting fms-related tyrosine kinase 3 (FLT3) as in inhibiting Mer. However, FLT3 has not been reported in human or mouse megakaryocytes or platelets. The results of Branchford *et al*. [1] clearly demonstrate that UNC2025 has antithrombotic characteristics both *in vitro* and *in vivo* in an arterial and venous thrombosis model, without increasing tail bleeding. These findings are in line with previous observations made in $Mer^{-/-}$ mice [5,8]. Interestingly, using an adapted tail bleeding protocol, Angelillo-Scherrer *et al*. [8] demonstrated that $Mer^{-/-}$ mice show a tendency to rebleed after initial cessation of

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bleeding, whereas initial bleeding times are unaltered. How this information can be applied to hemostasis in humans is still unclear. Importantly, UNC2025 inhibits phosphorylation of Mer, indicating a specific effect of the compound on its target receptor, and of Akt and Src. Although Akt and Scr are recognized downstream targets of Mer [3], a direct effect of UNC2025 on PI3K or Src, or via a platelet receptor other than Mer, cannot be ruled out completely. A key functional effect of signaling via Mer, and the other TAM receptors, is continued activation of $\alpha_{IIb}\beta_3$ [8,10]. Integrin $\alpha_{IIb}\beta_3$ activation is reversible, and impaired activation of this receptor causes instability of thrombi that are formed under flow *in vivo* (mouse) or *in vitro* (human and mouse), resulting in detachment of single platelets and small aggregates (reviewed in [15]). Similarly to observations made with (blood from) *Mer*$^{-/-}$ mice [8,10], treatment with UNC2025 resulted in reduced aggregate stability of human thrombi under flow. The combination of UNC2025 with low doses of a $P2Y_{12}$ inhibitor yielded a synergistic antiplatelet effect, both *in vitro* with human platelets, and *in vivo* in an arterial and venous thrombosis model. Intriguingly, this synergistic effect is not accompanied by an increase in tail bleeding time, such as is seen with high doses of $P2Y_{12}$ inhibitor [1]. Whether this combined treatment affects rebleeding has not been investigated yet.

How can a synergistic antithrombotic effect of UNC2025 on top of a low dose of $P2Y_{12}$ inhibitor without a concomitant increase in bleeding be explained? First, although Gas6 and $P2Y_{12}$ synergize at the level of Akt phosphorylation [10], platelet signaling pathways downstream of Mer and $P2Y_{12}$ may differ with respect to the temporal regulation of PI3K and Akt signaling and/or involve different downstream mediators. Support for different temporal regulation comes from the observation that Gas6–Gas6 receptor interaction becomes progressively more important at later stages, i.e. when the ADP pathway is reduced in activity or desensitizes [10]. Second, it appears that both $P2Y_{12}$ and Mer are also expressed in endothelial cells and leukocytes, at least at the mRNA level [3,16], indicating that multiple cell types may be involved in the thrombotic action of $P2Y_{12}$ and Mer. To exemplify this further, Gas6 has been demonstrated to upregulate tissue factor in endothelial cells upon vessel wall injury, leading to activation of the extrinsic coagulation pathway and to thrombus formation in mice [17]. Gas6 also triggers expression of the adhesion molecules ICAM-1 and VCAM-1 on endothelial cells, promoting platelet–endothelium and leukocyte–endothelium interactions [18], which suggests a role for Gas6 in platelet adhesion and activation under inflammatory conditions. Although *in vitro* flow chamber studies and experimental thrombosis models have provided valuable insights into the underlying mechanism of initial platelet interaction with activated endothelium, it remains obscure how thrombus formation and stabilization is regulated under these conditions [19].

How can the hemostatic effects of platelet Mer be dissected from those of endothelial Mer? A logical approach would be to use transgenic mouse models harbouring endothelial-specific or platelet-specific deletions of Mer. Alternatively, *Mer*$^{-/-}$ platelets could be transfused into mice that allow adoptive platelet transfer, e.g. human interleukin-4 receptor-α/glycoprotein Ibα-transgenic mice. The potential synergistic action of Mer and $P2Y_{12}$ could be investigated in these models by injecting *Mer*$^{-/-}$ or *P2yr12*$^{-/-}$ mice with, respectively, a $P2Y_{12}$ inhibitor or UNC2025 or, vice versa, by infusing inhibitor-treated platelets into donor mice. In addition, it will be of interest to investigate the effect of a platelet-specific or endothelial-specific deletion of Mer in mouse models of systemic inflammation, regardless of whether plasma Gas6 is elevated in these models or not. A potential complicating factor is that, besides Gas6, the following other ligands have been reported to bind to and activate Mer: protein S, Tubby, Tulp1, and galectin-3. Protein S serves as an anticoagulant predominantly by working as a non-enzymatic cofactor for activated protein C in the breakdown of activated factor V and activated factor VIII. Although human protein S has only weak or no affinity for the different human TAM receptors, there are reports indicating that human protein S affects Mer function in human macrophages (reviewed in [3,14]). So far, Tubby, Tulp1 and galectin-3 have been shown to be involved in Mer receptor-mediated efferocytosis (reviewed in [3]). Whether these ligands can affect hemostasis via Mer remains to be elucidated.

With regard to UNC2025, it is essential to further examine potential side effects, in particular those resulting from long-term treatment. So far, it has been shown that treatment with 75 mg/kg UNC2025 for several weeks leads to anemia and leukopenia in mice [20]. It is of note that a 25-fold lower dose of UNC2025 sufficed to fulfil an antithrombotic function in mice [1]. Given that UNC2025 is a Mer/FLT3 dual inhibitor, and that specific inhibition of FLT3 [21], but not of a loss of function of Mer [22], leads to similar defects in hematopoiesis as a high dose of UNC2025, it is likely that the reported side effects are mediated via inhibition of FLT3.

Together, the above findings indicate that the divergent roles of TAM receptors in hemostasis, inflammation and cell growth warrant careful study of potential adverse side effects when a TAM receptor is targeted as a therapeutic approach. However, they may also create opportunities to target pathologies that lie at the interface of these biological processes, e.g. inflammation-induced thrombosis. Patients who respond poorly to conventional antiplatelet therapy and have elevated plasma levels of platelet primers, such as Gas6, may benefit from treatment with a TAM inhibitor on top of standard medication. Moreover, if the synergistic effect of Mer and $P2Y_{12}$ on thrombosis, but not bleeding, holds true for humans, combined treatment in patients in need of antithrombotic medication but with a relatively high

bleeding risk may prove more beneficial than standard antiplatelet treatment.

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Disclosure of Conflict of Interests

The author states that she has no conflict of interest.

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