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Platelet granules in vascular integrity

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In this issue of Blood, Deppermann et al dissect the role of platelet granule secretion in maintaining vascular integrity during inflammation.1 The authors show that mice lacking platelet granule secretion do not bleed in skin or lung inflammation models. Lack of platelet release however resulted in increased brain hemorrhage after experimental stroke. The latter finding is of clinical importance when designing novel therapies to improve stroke outcome.

It is well known that platelets are crucial for stopping bleeding. Platelets prevent excessive posttraumatic blood loss at sites of vascular injury by forming a platelet plug. Upon exposure of the subendothelial extracellular matrix, platelets are recruited to the site of injury and become activated, resulting in firm adhesion and subsequent platelet aggregation. The molecular mechanisms underlying the formation of a hemostatic platelet plug are relatively well understood: upon exposure of the subendothelial matrix, platelets either interact directly with matrix proteins (eg, via glycoprotein VI [GPVI] and αIIbβ3 to collagen) or bind to von Willebrand factor (VWF) that is deposited at the site of injury. Transient interactions between platelet GPIb and VWF support platelet tethering at sites of high shear stress. Firm adhesion and subsequent aggregation is mediated by activated integrin receptors such as αIIbβ3. G-protein–coupled receptors mediate activation signals after being triggered by soluble agonists such as thrombin, thromboxane A2, and adenosine 5′-diphosphate, which reinforce thrombus propagation.

Interestingly, newer insights reveal that platelets also safeguard a different form of hemostasis by maintaining vascular integrity during acute inflammation.2 It was recently shown that single platelets seal vascular breaches caused by neutrophils.3 In contrast to our understanding of vascular injury-induced thrombus formation, much less is known about the mechanisms used by platelets to prevent inflammation-induced hemorrhage. Intriguingly, the process by which platelets maintain vascular integrity at the site of inflammation is independent from the ability of platelets to form a hemostatic platelet plug. Indeed, neither the adhesion receptors GP Ib and αIIbβ3, nor signaling via G-protein–coupled receptors are necessary to maintain vascular integrity in inflamed organs.4,5 The immunoreceptor tyrosine-based activation motif receptors GPVI and CLEC2 on platelets have been identified as crucial mediators supporting vascular integrity.3,5 However, the exact triggers that induce platelet signaling and the downstream effector mechanisms involved in the prevention of inflammatory bleeding remain unclear. Platelet components released from intracellular storage granules have been suggested to be implicated in this process.6

To address the role of platelet granule content in maintaining vascular integrity in inflammation, Deppermann et al generated Unc13d−/−/Nbeal2−/− mice.1 Platelets from these mice are unable to secrete their α- or dense-granule content. The authors used these mice in models of lung inflammation, skin inflammation, and brain infarction. Similar to previous studies, intradermal hemorrhage was observed in platelet-depleted wild-type (WT) mice at the site of inflammation. Strikingly, no bleeding was observed in the inflamed skin of Unc13d−/−/Nbeal2−/− mice. Analogous results were observed in lung inflammation. These experiments show that release of α or dense granules is not necessary to maintain vascular integrity at sites of acute inflammation in skin and lung. Much different results were however obtained in the stroke model used by the authors. Indeed, when subjected to transient middle cerebral artery occlusion, Unc13d−/−/Nbeal2−/− mice were prone to intracranial bleeding in the infarcted areas. Cerebral hemorrhage in these mice resulted in a significantly increased mortality compared with WT animals. In an elegant approach using platelet transfection experiments, the authors showed that the observed effects of combined Munc13-4 and Nbeal deficiency were related to the platelet-specific secretion effects and not to potential defects in other cells.

The results from Deppermann et al are important in 2 ways. First, this study shows that platelets use different pathways to ensure hemostasis in different inflammatory settings and vascular beds. Second, the results demonstrate that platelet granule release is important to safeguard hemostasis during stroke injury. The latter insight might become particularly relevant for the development of novel treatment of ischemic stroke. Maintaining cerebral hemostasis during a stroke is of high clinical relevance because intracranial bleeding often leads to aggravation of the disease state and increase of mortality. Strategies to prevent or treat acute ischemic stroke should not increase the risk of cerebral bleeding. In this context, anti-thromboinflammatory therapeutics have shown promising preclinical results.7

Thromboinflammation causes progressive ischemic brain damage via complex pathways that include early platelet adhesion and activation but not platelet aggregation.7 Importantly, the release of α or dense granules also contributes to thromboinflammatory brain injury.8,9 Correspondingly, in the current study, Deppermann et al observed reduced infarct volumes and fewer neurological deficits in those Unc13d−/−/Nbeal2−/− mice that did not die of intracranial hemorrhage. Hence, although preventing platelet granule release might seem an attractive strategy to reduce

7. Gustafsson B, Bogdanovic G. Specific viruses were not detected in Guthrie cards from children who later developed leukemia. Pediatr Hematol Oncol. 2007;24(8):607-613.

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**Platelet polyphosphate: the long and the short of it**

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In this issue of *Blood*, Verhoef et al provide evidence that platelets contain polyphosphate polymers of sufficient size to promote activation of factor XII (FXII), thereby addressing a long-standing enigma as to the potential contribution of platelet polyphosphate to thrombosis.

Platelet dense granules contain inorganic polyphosphate (polyP) and divalent metal ions, which are released upon platelet activation. PolyP consists of varying-length chains of negatively charged phosphate units. A potent procoagulant, polyP initiates coagulation by binding FXII and promoting its autoactivation. The conundrum is that polyP secreted by platelets consists of 60 to 100 phosphate units and is a less efficient activator of FXII than are polyP polymers consisting of 200 or more phosphate units, such as those found in microorganisms. How then does platelet polyP activate FXII? Verhoef and colleagues provide a potential answer. They show that the polyP released from platelet-dense granules aggregates into nanoparticles that accumulate on the platelet surface and are of sufficient size to promote FXII activation.

If correct, they provide a plausible explanation for the bleeding diathesis that occurs in patients with Hermansky-Pudlak syndrome, whose platelets are deficient in dense granules and contain lower concentrations of polyP than do normal platelets. Despite these observations, however, prior to the work of Verhoef and colleagues, it remained unclear how platelet polyP could activate FXII.

While investigating a probe for detecting platelet-bound polyP, this group previously observed that exogenous long-chain polyP binds to platelets, whereas short-chain polyP does not. They also showed accumulation of endogenous polyP on the surface of activated platelets. In the current study, Verhoef and colleagues demonstrate that, whereas the total polyP in platelet lysates promotes contact activation in a FXII-dependent manner, the soluble polyP fraction does not. These results suggest that platelets contain long-chain polyP, but do not necessarily release it when they are activated. Using confocal and electron microscopy, Verhoef et al visualized spherical polyP nanoparticles ranging in diameter from 100 to 200 nm on the granulomere of spread platelets. Formation of these nanoparticles appears to be dependent on polyP precipitation by divalent metal ions, because exposure of platelets to ethylenediaminetetraacetic acid (EDTA) abrogated their accumulation. Furthermore, Ca²⁺ addition to short-chain
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