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Platelets: connecting clotting and lysis

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In this issue of *Blood*, Whyte et al describe how under flow conditions, phosphatidylserine-expressing platelets modulate the lysis of whole blood clots by providing direct and indirect binding sites for plasminogen.[1]

Platelets are not the first thing to come to mind when one thinks about fibrinolysis. However, platelets contain several fibrinolytic factors packaged in their α granules, including fibrinogen, plasminogen, plasminogen activator inhibitor-1, α_2-antiplasmin, and thrombin activable fibrinolysis inhibitor, or procarboxypeptidase U. Therefore, platelets certainly have the potential to modulate fibrinolysis by controlled release of these substances.

Researchers have been investigating the role of platelets in fibrinolysis for many years. Platelets were first reported to promote plasminogen activation in the mid-1980s, and studies then focused on how this occurs. Miles and Plow[2] demonstrated that fibrin matrix formation on the platelet surface enhances plasminogen binding. More recently, Baeten et al[3] showed that platelet-bound plasminogen promotes fibrinolysis by converting single-chain urokinase plasminogen activator into two-chain urokinase, which then activates plasminogen in a reciprocal fashion. However, all of these experiments were conducted using purified reagents and under static conditions.

Whyte et al[1] have taken things to the next level by elegantly demonstrating how platelets modulate fibrinolysis in whole blood under flow conditions. Thrombi were formed by perfusing whole blood supplemented with fluorescently labeled platelets, fibrinogen, and plasminogen over a glass coverslip coated with spots containing collagen or tissue factor. This is an innovative setup because it allows for mimicking blood flow in a vessel while monitoring the clotting and/or lysis in real time. Plasminogen binds to platelets before fibrin matrix formation, but binding is enhanced when fibrin is present. Therefore, consistent with the previous work,[4] there are 2 distinct mechanisms of plasminogen binding: plasminogen can bind directly to platelets or it can bind indirectly in a fibrin(ogen)-dependent manner. Furthermore, indirect binding appears to be the dominant mechanism. Also, when heparin was included in the perfused whole blood system to prevent thrombus accretion, clot degradation was enhanced.

Shankaran et al[5] showed that platelets activated under high shear conditions express more phosphatidylserine (PS) on their surface than platelets activated under static conditions. In addition, Robbie et al[6] demonstrated that thrombi formed under high shear conditions contain more platelets than those formed at low shear. Together, these observations suggest that the presence of shear stress during thrombus formation results in a clot that is rich in platelets that have enhanced PS expression on their surface, thus creating an even greater procoagulant environment compared to clots formed under low shear. What comes as a surprise is that despite the highly procoagulant nature of these thrombi, PS also appears to augment plasminogen binding to the platelet surface in a process that requires $\alpha_{IIb}\beta_3$ activation and fibrin polymerization. In support of this, Whyte et al demonstrate that the enhancement of fibrinolysis can be attributed to flow alone, inferring that greater PS expression from high shear leads to enhanced plasminogen binding and, thus, fibrinolysis. It was determined that PS-positive platelets were able to localize plasminogen binding to a protruding "cap" that had greater plasminogen activation properties in a fibrin(ogen)-dependent manner compared to PS-negative platelets. Taken together, these observations provide insight as to how coagulation and fibrinolysis are intimately connected and highly regulated.

Even with the development of methods such as thromboelastography, investigating clotting and fibrinolysis in whole blood systems remains challenging. This is especially true for fibrinolysis because it is a secondary process that follows clotting and, thus, tends to have larger variability in the quantification parameter values (eg, time to 50% lysis). The methods used by Whyte et al allow for investigation not only of whole blood systems but, more importantly, of whole blood under flow that can be observed in real time and with high reproducibility. Their results shed light on the mechanisms involved in initiating and modulating fibrinolysis in vivo.

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