

inside **blood** commentary

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Comment on Stritt et al, page 219

The role of RIAM in platelets put to a test

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In this issue of *Blood*, Stritt et al show that platelet functions dependent on integrin activation are unimpaired in mice lacking the Rap1-GTP-interacting adaptor molecule (RIAM).¹

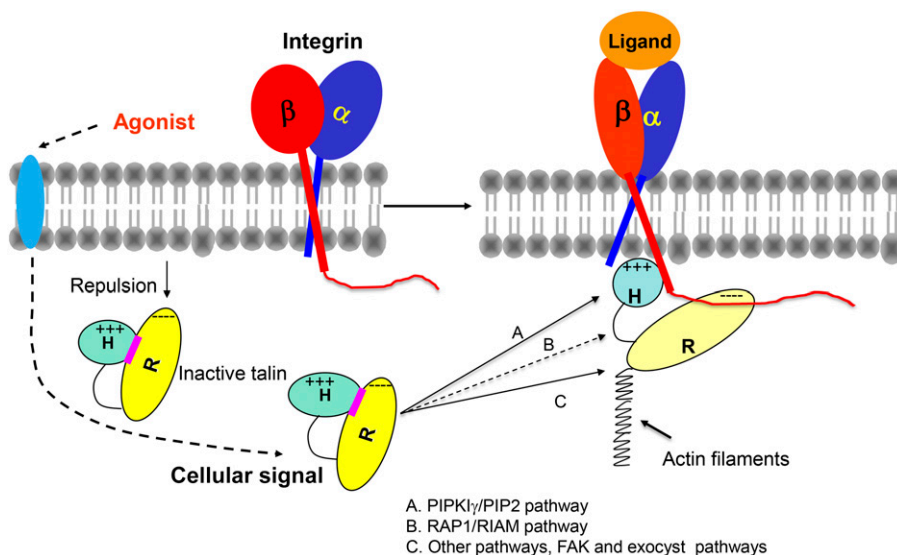
RIAM, a member of the Mig-10/RIAM/lamellipodin family,² contains domains that bind Rap1-GTP, phosphotylinositol-4,5-bisphosphates, and talin, a large cytoskeletal protein that is required for activation of integrin adhesion receptors.³ These binding sites in RIAM were suggested to enhance recruitment of talin to membranes and to integrins and thereby increase integrin activation and the consequential cellular

responses, including many platelet responses. Indeed, overexpression of RIAM modestly enhances and knockdown of RIAM inhibits activation of the major platelet integrin, α IIB β 3, in heterologous cells.^{4,5} Based on such observations, RIAM has been thought to be a crucial component of the inside-out signaling machinery that leads to integrin activation.^{4,5} Other studies have suggested that RIAM influences cell spreading and

cytoskeletal organization (ie, RIAM also plays a role in outside-in signaling).^{2,6} Accordingly, there is evidence that RIAM mediates the bidirectional signaling across integrins. Thus, the study by Stritt et al, showing that platelet integrin function is apparently normal in RIAM-null mice, comes as a surprise and is potentially paradigm shifting.

The RIAM-null mice were created by standard homologous recombination technology. Although platelets were the focus of the study, the mice displayed no overt phenotype, were fertile, and had normal blood cell counts, which is an important observation because RIAM is abundant in many cells including other hematopoietic cells.² No RIAM protein was detected in platelets from RIAM-null mice, whereas RIAM was readily detected in platelets from wild-type mice. As for integrin α IIB β 3 activation-dependent responses, the authors tested the binding of monoclonal antibody JON/A and fibrinogen, reagents that bind selectively to the activated integrin, and found these to be completely normal. For functional responses, platelet aggregation was assessed. Aggregation in response to various doses of different agonists was not different for RIAM-null and wild-type platelets, with some analyses done with washed platelets and others done in plasma. In short, responses dependent on activation of α IIB β 3, the very integrin shown to be affected by alteration in RIAM expression levels in heterologous cells, were unaffected by RIAM deficiency in mouse platelets.

Function of β 1 integrins in platelets also appeared to be similar in wild-type and RIAM-null platelets. Binding of a β 1 activation-specific monoclonal antibody and adhesion to collagen under flow, which involves a β 1 integrin, were similar for the null and wild-type platelets. Thus, although not extensively documented, this preliminary survey suggests that the β 1 integrins are also fully functional in the absence of RIAM.



Pathways for talin-mediated activation of integrins. In the unstimulated platelet, integrin α IIB β 3 exists in a resting state (does not bind ligand with high affinity) and talin exists in the cytosol in an autoinhibited state in which interaction between its rod (R) domain and its head (H) domain occludes the integrin-binding site in the head domain. Three pathways are known to relieve the autoinhibition of talin, allowing its recruitment to the cell membrane via a push-pull charge-dependent mechanism⁶ and to the integrin β subunit. The paper by Stritt et al¹ indicates that the RIAM is dispensable for talin-mediated activation of integrin and the responses mediated by activated and occupied integrin α IIB β 3.

Turning their attention to outside-in signaling across integrins, the authors evaluated platelet adhesion and spreading on fibrinogen and platelet-mediated clot retraction. These responses are all dependent on outside-in signaling across integrin α IIB β 3, and all were similar with platelets from RIAM-null and wild-type mice.

Of course, a particularly crucial issue is whether the absence of RIAM affects platelet integrin α IIB β 3 activation-dependent responses in vivo. The authors address this question by assessing tail bleeding time, an assessment of hemostasis, and time to occlusion following FeCl₃-induced injury to the mesenteric arterioles, an assessment of thrombus formation. Both of these assays are known to have limitations but are among the most widely used models to evaluate hemostasis and thrombus formation in the mouse. In both models, the RIAM-null and wild-type mice did not differ significantly. Thus, two of the most relevant physiological/pathological responses mediated by platelets and their integrins appear to be fully functional in the absence of RIAM.

The compilation of these findings leads to a clear conclusion: RIAM is not essential for integrin activation in platelets or for platelet responses dependent on integrin activation. How is this conclusion reconciled with previous data showing that manipulation of RIAM levels in model cells influences activation of integrins, including integrin α IIB β 3? Several possible explanations can be considered. As currently envisioned, integrin activation depends on the binding of talin via its head domain to the cytoplasmic tail of the integrin β subunit.³ Talin exists in the cytosol in an autoinhibited state, in which its rod domain occludes the integrin-binding site in its head domain.^{7,8} RIAM was envisioned as being involved in activating talin for binding to integrin and in recruiting talin to the membrane, a step needed for appropriate orientation of talin's head for integrin activation.⁵ However, talin activation and recruitment can be achieved by multiple mechanisms (see figure), and RIAM provides only 1 route to the end of integrin activation. Thus, 1 plausible explanation for the observations is that these other pathways are sufficient in platelets, making RIAM dispensable for integrin activation-dependent responses. A second possibility is that other MLR

family members or other Rap1 effectors may fulfill the integrin activating functions of RIAM in platelets. With the absence of a platelet phenotype in RIAM-null mice but substantial defects in integrin-mediated responses in Rap1-deficient mice,⁹ it is clear that RIAM-independent mechanisms exist for Rap1 to exert its effects on integrins. A third possibility is that the functions of RIAM may display species specificity in terms of the integrins, talins, or additional cofactors needed to display its integrin-activating function. The RIAM pathway may also become important in the contribution of platelets to disease development, a scenario that has yet to be explored.

The study by Stritt et al provides the first step and identifies a key tool, the RIAM-null mouse, in defining the functions of RIAM. Immediate questions needing to be addressed are whether integrin functions in other hematopoietic cells are compromised in the RIAM-null mice and what phenotypes manifest when these mice are subjected to various challenges.

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

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● ● ● CLINICAL TRIALS & OBSERVATIONS

Comment on Cannegieter et al, page 229

Superficial venous thrombosis: deeper than meets the eye?

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In this issue of *Blood*, Cannegieter et al use the Danish National Patient Registry to report on the high incidence of venous thromboembolism (VTE), mortality, and arterial thrombosis following a diagnosis of superficial vein thrombosis (SVT). Remarkably, individuals with SVT had nearly a 14% risk of VTE over 10 years, with a 3.3% risk of VTE in the 3 months following the SVT.¹ Although during the 10 years of follow-up this translated into an 8-fold increased risk of VTE, the risk was much higher within the 3 months following an SVT (71-fold increased risk of VTE). The hazard ratios of arterial events and death over the entire follow-up were more modest (around 1.2-1.3), however, with noticeably increased risk within 3 months of the SVT event with a 1.5- to 3.5-fold increased risk.

Thrombosis is a family of diseases, with common manifestations such as VTE and SVT and rarer ones such as cerebral sinus thrombosis and splanchnic vein

thrombosis. Although there are common themes and risk factors for each site of thrombosis, risks and benefits of treatment as well as individual risk factors