in tissues. These studies also support PD-1 blockade as an effective tool to put in the toolbox for developing HIV CAR T-cell immunotherapies. In sum, these important studies advance the HIV immunotherapy field toward the ultimate goal of curing HIV infections.

Conflict-of-interest: P.J.S. is cofounder and the chief scientific officer of MarPam Pharma LLC. ■

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PLATELETS AND THROMBOPOIESIS

Comment on Bhatlekar et al, page 1760

Untying knots to make more platelets

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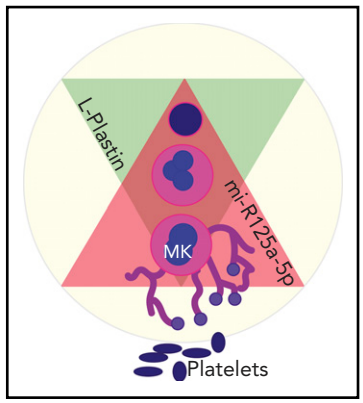
In this issue of *Blood*, Bhatlekar et al[1] have identified microRNA (miRNA) *miR-125-5p* as a regulator of L-plastin, an actin bundling protein, and show how L-plastin levels control platelet formation by megakaryocytes.

When blood vessels are damaged, blood loss has to be stopped. This is the function of small blood cells called platelets. These cells form aggregates and create a plug that sticks to the wound to prevent bleeding. You want to have the right amount of this platelet "glue" though, since too few or too many platelets give rise to conditions such as thrombocytopenia and thrombocytosis, which increase the risk of serious bleeding and clot formation, respectively. Platelet production is an intriguing process. It starts in the bone marrow, where the platelet progenitor cells, called megakaryocytes, develop and increase in size during their maturation process. Finally, numerous platelets are released from each megakaryocyte via the formation of elongated branches, called proplatelets, in a process that requires extreme changes to the cell's structure and cytoplasmic organization. These changes in cell shape and motility depend on the actin cytoskeleton. The importance of the cytoskeleton in platelet biogenesis is illustrated by the reduction in platelet formation in conditions characterized by mutations of cytoskeletal components such as tubulin[2] and myosin.[3] Whether changes in the cytoskeleton can increase platelet formation is not known. Gaining a better understanding of this aspect of platelet formation is of substantial clinical and physiological importance.

Bhatlekar et al, in this issue of *Blood*, identified miRNA *miR-125a-5p* as a positive regulator of platelet production by skillfully correlating their previous findings on miRNAs associated with platelet count in 154 healthy donors[4] with miRNA profiling in (i) purified mature bone marrow megakaryocytes, (ii) platelets from the same bone marrow donors, and (iii) megakaryocytes derived from cord blood cells in the laboratory. Following a similar rigorous approach, the authors narrowed down the thousands of potential *miR-125a-5p* targets and identified L-plastin as one of the main factors downregulated by *miR-125a-5p* in megakaryocytes. L-plastin (*LCP1*) is an actin-binding protein that links actin filaments and stabilizes parallel strands. This paper shows that lower levels of L-plastin facilitate platelet formation (see figure).

The authors first established that knockdown of *miR-125a-5p* led to lower platelet formation. Conversely, overexpression of *miR-125a-5p* boosted platelet numbers. They then confirmed the inverse relationship between *miR-125a-5p* and L-plastin expression by increasing and decreasing *miR-125a-5p* levels in human megakaryocytes and mouse bone marrow. To understand the role of L-plastin in platelet production, Bhatlekar et al used CRISPR-Cas9 gene editing technology to lower L-plastin levels and show that this reduction considerably increased proplatelet formation and proplatelet branching (which positively correlates with platelet formation). Megakaryocytes require actin-containing structures called podosomes to degrade extracellular matrix, which is necessary for delivery of platelets into the circulation.[5] In this study, the authors demonstrate that L-plastin knockdown markedly increased the number of podosomes per megakaryocyte. This is likely to contribute to enhanced platelet release in vivo. Together, these observations provide evidence of a new nexus in platelet production characterized by the inverse relationship between *miR-125a-5p* and L-plastin.



Negative association between L-plastin and human platelet numbers proposed by Bhatlekar et al in this issue of *Blood*. Levels of *miR-125a-5p* (magenta triangle) increase during megakaryopoiesis. *miR-125a-5p* targets and downregulates expression of L-plastin (lime green triangle), thus enhancing proplatelet formation by megakaryocytes (MK).

Of course, there are interesting remaining questions. The authors observed a stronger phenotype upon manipulation of *miR-125a-5p* than following targeting of L-plastin. Presumably, *miR-125a-5p* downregulates additional targets in megakaryocytes that, coincidentally, also lead to increased platelet production. How L-plastin regulates podosome development, whether increased podosome numbers are relevant for in vitro proplatelet formation, and the function of L-plastin in the megakaryocyte invaginated membrane system are yet to be determined. Development of megakaryocyte-specific *miR-125a-5p* and/or L-plastin knockout murine models would be invaluable, since conflicting results have been reported in existing global knockout and overexpression models.[6,7]

These findings have potential implications for disorders of platelet number, since dysregulation of the *miR-125a-5p*/L-plastin axis could be a factor in the pathogenesis of these conditions. In this paper, the authors present proof of principle that judicious manipulation of *miR-125a-5p* could be used to balance abnormal platelet numbers. In addition, the long-term ambition of producing sufficient platelets in vitro for autotransfusion would benefit from strategies that might optimize platelet yield per megakaryocyte, such as overexpression of *miR-125a-5p* or knockdown of L-plastin. Overall, the authors offer a novel insight into the captivating process that fragments megakaryocytes, the largest cells in bone marrow, into the smallest cells in the circulation.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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THROMBOSIS AND HEMOSTASIS

Comment on DeHelian et al, page 1773

Let's "brake" it down

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After decades of dissecting the signaling pathways that trigger and amplify platelet activation, we still know very little about the regulatory mechanisms that limit and control these processes. In this issue of *Blood*, DeHelian and colleagues shed light on the importance of RGS10 and RGS18, 2 molecular brakes of the regulator of G protein signaling (RGS) family, in modulating platelet activation and platelet number.[1]

Effective hemostatic plug formation at sites of injury relies on the rapid recruitment of platelets from the bloodstream and on their near-immediate conversion from a nonadhesive to a proadhesive state. Nearly all soluble agonists generated at sites of injury stimulate platelets via heterotrimeric G protein–coupled receptors (GPCRs). Heterotrimeric G proteins consist of 3 subunits, α, β, and γ, strategically inserted in the inner leaflet of the plasma membrane to relay signals coming from the extracellular space. GPCRs engagement displaces the GDP from the Gα subunit and allows for the immediate loading of the more abundant guanosine triphosphate (GTP) onto the nucleotide-free G protein. This on-switch mechanism provides a perfect system to transduce signals on a millisecond scale required for platelet adhesion and 3-dimensional thrombus growth under shear stress conditions. However, it must be tightly controlled to avoid thrombosis or thrombocytopenia that may result from increased clearance of activated platelets.

In the middle to late 1990s, studies in yeast and *Caenorhabditis elegans* led to the discovery of a new family of regulators of G-protein signaling capable of reducing the amplitude and duration of GPCR signaling by increasing the rate of GTP hydrolysis and returning the G protein to the off state. Fifteen years later, the Brass group made the exciting observation that these RGS proteins may be important negative regulators of GPCR signaling also in platelets, since mice expressing a mutant Gαi subunit unable to bind RGS proteins displayed enhanced platelet function in vitro and in vivo.[2] However, as they and others went on to carefully characterize the contribution of individual RGS isoforms to platelet function,[3-5] the role and importance of RGS proteins were put into question. As expected, genetic ablation in mice of either one of the major RGS isoforms expressed in platelets, *Rgs10* or *Rgs18*, shortens bleeding times as well as thrombus occlusion times in vivo, but the phenotype of the single knockouts is milder than that of the Gαi mutant and of other mouse models lacking established molecular brakes of platelets, such as the ITIM receptor G6b-B[6] and the RAP1-GTPase activating protein RASA3[7] (see figure). One possible explanation for the mild phenotype is of course the redundancy between RGS isoforms; thus, the authors set out to investigate the phenotype of mice lacking both *Rgs10* and *Rgs18*.

With this study, DeHelian et al demonstrate beyond further doubt that RGS10 and RGS 18 have an important and redundant role in dampening agonist-induced platelet activation and thrombus growth at sites of vascular injury. Indeed, deficiency in both RGS isoforms leads to an exaggerated platelet accumulation and frequent occlusion of injured vessels, thus supporting the idea