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.

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THROMBOSIS AND HEMOSTASIS

Comment on DeHelian et al, page 1773

Let's "brake" it down

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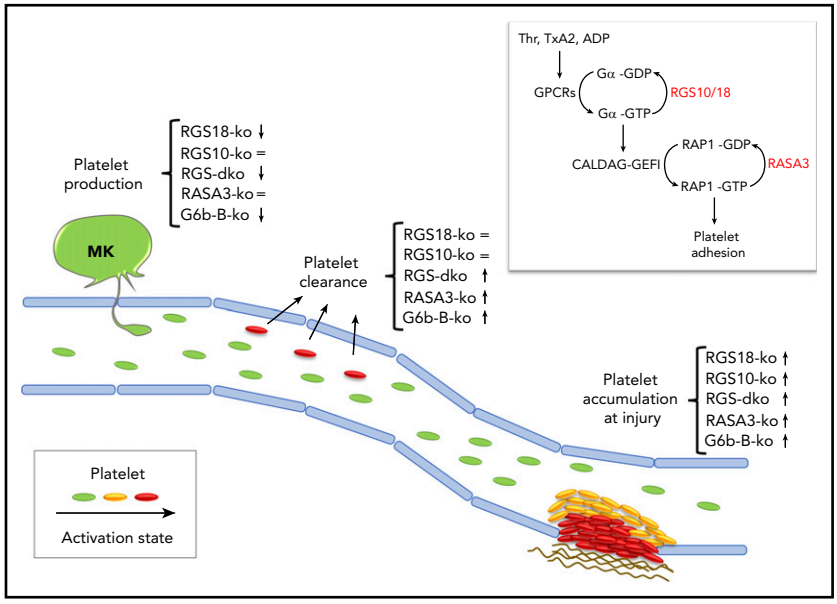
After decades of dissecting the signaling pathways that trigger and amplify platelet activation, we still know very little about the regulatory mechanisms that limit and control these processes. In this issue of *Blood*, DeHelian and colleagues shed light on the importance of RGS10 and RGS18, 2 molecular 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



In vivo phenotype of mice lacking RGS10/18 or other platelet molecular brakes. Key features of the in vivo phenotype of mice lacking critical molecular brakes of platelets are (1) increased platelet accumulation at sites of injury, (2) increased platelet clearance, and (3) thrombocytopenia. Double deficiency of RGS10 and RGS18 results in an exaggerated thrombus growth and in a mild thrombocytopenia, which is in part due to increased platelet clearance, and in part due to a nonredundant contribution of RGS18 to the regulation of platelet production. The phenotype of other molecular brakes, RASA3 and G6b-B, is shown for comparison. Symbols indicate whether a knockout mouse (ko) displays an increased (↑), decreased (↓), or unaltered (=) phenotype. The inlet shows the 2 main G protein switches that control rapid agonist-induced platelet adhesion: (1) RGS10/18 negatively regulates the heterotrimetic G proteins coupled to surface receptors (GPCRs) stimulated by soluble agonists such as thrombin (Thr), thromboxane A2 (TxA2), and adenosine diphosphate (ADP); (2) RASA3 negatively regulates the monomeric G protein RAP1, critical regulator of integrin-mediated adhesion. MK, megakaryocyte.

that the mild phenotype observed in the single knockouts was just due to redundancy between the 2 isoforms. One might wonder, what is the physiological relevance of 2 related proteins that basically compensate for each other? In biological systems, redundancy provides a safety net that ensures that important signaling steps take place no matter what. Thus, it is not surprising to find redundant proteins in a crucial signaling node controlling the balance between platelet activation and platelet inhibition.

Brakes are necessary not only to limit thrombus growth but also to prevent unwanted activation of circulating platelets in the absence of an injury. For this reason, mice lacking molecular brakes of platelet activation typically display increased markers of platelet activation even in the absence of stimuli, increased platelet turnover, reduced platelet half-life, and thrombocytopenia due to increased clearance. This is not entirely the case for RGS proteins. Neither of the single knockout mice show evidence of premature platelet clearance. *Rgs10*$^{-/-}$ mice have a normal platelet count/size, and *Rgs18*$^{-/-}$ mice display a mild thrombocytopenia (~15% reduction) caused by defects in megakaryocyte function. A phenotype becomes apparent only when both *Rgs* genes are deleted, again because of redundancy. The double knockout mice are characterized by circulating platelets with exposed TLT-1, a marker of secretion, an increased proportion of reticulated platelets, indicative of augmented platelet turnover, and a 40% reduction in platelet count. However, the role of RGS10 and RGS18 in limiting preactivation of circulating platelets is presumably modest, since genetic ablation of other established molecular brakes, such as RASA3, leads to more dramatic platelet count reductions.[7] This different phenotype is likely due to different regulatory mechanisms. In quiescent circulating platelets, while RASA3 is always active, RGS10 and RGS18 are trapped into a complex with spinophilin that prevents them from sending tonic inhibitory signals to GPCRs in the absence of stimuli. Why are they different though? Are there other molecular brakes restraining GPCR signaling in unstimulated platelets? Or is it that RAP1 proteins are more likely than GPCRs to become randomly activated in circulation, owing to the fact that the RAP1 activator CalDAG-GEFI is extremely sensitive to intracellular calcium fluxes? More basic scientific work is needed to answer these important questions.

Finally, this study confirms that RGS18, but not RGS10, contributes to the regulation of platelet production. The authors were not able to pin down the mechanism underlying this observation. Thus, further studies are needed to dissect how this molecular brake supports normal platelet production and whether mutations in *Rgs*18 are associated with congenital thrombocytopenia in humans as is the case for the ITIM receptor G6b-B.[8]

In summary, DeHelian and colleagues identify both redundant and nonredundant roles of the 2 major isoforms of the RGS family, RGS10 and RGS18, in modulating platelet activation and platelet number. Characterization of these 2 molecular brakes may have important implications to better understand pathological conditions in which stimulatory and inhibitory signaling pathways are unbalanced.

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LYMPHOID NEOPLASIA

Comment on Saini et al, page 1786

TKI vs relapse after HSCT: is the jury still out?

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In this issue of *Blood*, Saini and colleagues report that administration of BCR-ABL1 active tyrosine kinase inhibitors (TKIs) after allogeneic hematopoietic stem cell transplantation (HSCT) prevents relapse and improves relapse-free survival in patients with Philadelphia-positive acute lymphoblastic leukemia (Ph$^+$ ALL).[1] Their retrospectively collected data represent experience from a large single center and reinforces the conclusion reached by a limited number of previous studies that TKI maintenance after HSCT improves patient outcome.[2-5] Surprisingly, TKIs have so far not been widely recognized to play an important role after HSCT, even though their central role in first-line therapy for Ph$^+$ ALL has been recognized for nearly 2 decades. Moreover, relapse accounts for approximately half of treatment failures after HSCT and affects ~25% of transplanted patients, making this a significant clinical problem. Ironically, the type of randomized trial that could have generated conclusive evidence supporting the use of TKIs after HSCT was prevented by the long-standing conviction that for ALL matched related donor (MRD) is a legitimate trigger for therapeutic intervention. Coupled with the knowledge that MRD after HSCT is a harbinger of relapse,[6] and overt relapse after HSCT is nearly impossible to salvage, a nonintervention control or a control deemed ineffective such as donor-lymphocyte infusions (DLIs) was considered unethical. The only prospective randomized trial published to date compared prophylactic with preemptive (MRD-triggered) administration of imatinib and demonstrated that both treatment arms were equivalent and superior to historical experience.[3] Although this trial also allayed concerns about possible detrimental effects of starting imatinib early after HSCT (eg, due to drug–drug interactions or delayed hematopoietic or immune recovery), the possibility remained that the superior outcome with TKIs compared with historical controls was merely due to patient selection and improved supportive care. This caveat persisted despite data from subsequent single-arm trials and retrospective analyses supporting the central tenant that TKIs should become part of a standard posttransplant management.

Despite the limitations inherent in retrospective analyses, the study by Saini et al adds considerable support to the concept of posttransplant TKIs by providing data on one of the largest patient cohorts studied to date. Against the backdrop of previous data based almost exclusively on imatinib, this study additionally demonstrates that second- and third-generation TKIs are likewise of benefit when given as posttransplant maintenance. Interestingly, the more advanced TKIs appeared equally effective to imatinib in reducing the relapse rate overall but were superior when TKI use was triggered by MRD. Moreover, patient outcome was significantly better with prophylactic than with MRD-triggered TKIs, a finding that differs from results of the prospective randomized trial by Pfeifer et al mentioned above.[3] Likely reasons for these differences are a larger proportion of poor-risk patients in the MRD-triggered cohort and higher median MRD levels when TKIs were started. The latter point is suggested by the 4-weeks longer median time to commencing TKI administration in the MRD-triggered groups, ample time for the disease burden to increase in a disease known for rapid disease kinetics. The authors tried to address this bias by restricting their analysis to patients in CMR who were alive and in CMR 3 months after HSCT, but the number of evaluable patients meeting these criteria was small and it is doubtful whether this observed inferiority of an MRD-triggered approach can be extrapolated to all clinical situations. It is worth noting that the median time to molecular relapse in the study by Pfeifer et al was only 4 weeks, and time to MRD-triggered TKIs was 2.3 months compared with 3.2 months in the study reported here.[1,3] Physicians should therefore be acutely aware of the short time window for intervention afforded to them by the nature of this disease and base decisions on which strategy to pursue, prophylactic or MRD triggered, on logistical considerations such as turnaround time for results and probably also MRD levels at the time of HSCT. These were unfortunately not available in the study by Saini et al.

Another topic of profound practical significance for patient management is that of optimal duration of TKI administration after transplant. In their paper, the authors suggest this should be at least 2 years and up to 5 years, which is longer than the time periods chosen in other studies in which ~ 70% of patients discontinued TKIs prematurely. TKIs are known to be subjectively less well tolerated when given after HSCT than before, even though severe toxicity is distinctly unusual. Although a 2-year treatment period appears operationally reasonable, physicians should be cognizant of the need to reassure and motivate their patients to continue taking the TKIs. Inability to ensure compliance should lead to more frequent MRD monitoring.

Has the verdict on TKI maintenance after HSCT been reached? The balance of