

Platelet Fc γ RIIA signaling: new clues for HIT

Alice Assinger MEDICAL UNIVERSITY OF VIENNA

In this issue of *Blood*, Zhou et al provide novel insights into Fc gamma type 2 receptor A (Fc γ RIIA)-mediated platelet activation. By identifying a negative regulator of Fc γ RIIA signaling, which is influenced by microRNA miR-148a, they present the first evidence of modulation of platelet function by microRNAs in vivo. They show that inhibition of miR-148a prevents thrombosis after immune complex-induced platelet stimulation in vivo.¹

Immune-mediated heparin-induced thrombocytopenia (HIT) is an atypical immune complication of heparin treatment that affects 1 in 5000 hospitalized patients. Approximately 50% of these patients develop

thromboembolic complications.² HIT is induced by antibodies that recognize complexes of heparin and platelet factor 4, which trigger platelet activation after binding to platelet Fc γ RIIA. Interestingly, platelet

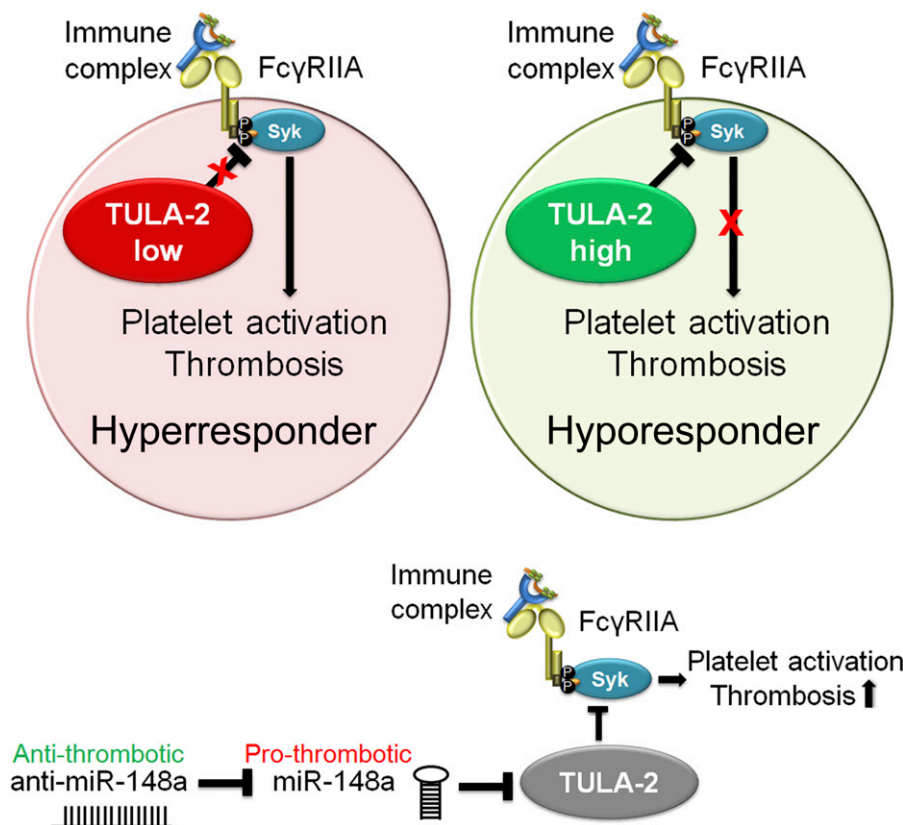
activation in response to immune complex binding differs significantly between individuals. One possible explanation for these inter-individual variations could be based on the structure and expression levels of Fc γ RIIA, which determines immune complex binding capacity. However, Zhou et al show that the differences in Fc γ RIIA activity remain largely unexplained by Fc γ RIIA expression levels or by a polymorphism that affects immunoglobulin G subclass binding affinity. In particular, individuals who are heterozygous for the principal Fc γ RIIA polymorphism showed unpredictable platelet responses. This suggests that other factors determine platelet reactivity in response to Fc γ RIIA-mediated activation.

To address this possibility, Zhou et al¹ take a novel approach by investigating potential negative regulators of Fc γ RIIA signaling that could explain the differences between individuals. They identify a critical role for platelet T-cell ubiquitin ligand-2 (TULA-2) as a negative regulator of Fc γ RIIA-mediated signaling in platelets. Platelets with low expression levels of TULA-2 strongly respond to immune complexes, whereas high levels of TULA-2 protect from platelet aggregation in response to immune complex binding to Fc γ RIIA (see figure).

TULA-2, which counteracts the phosphorylation of Syk,³ is a key protein in the downregulation of receptor-mediated signal transduction in platelets in vitro and in vivo. Zhou et al showed that TULA-2 also regulates Fc γ RIIA-mediated signaling in platelets.

Interestingly, Zhou et al find TULA-2 to be negatively regulated by miR-148a (see figure), one of the 750 microRNAs found within platelets.⁴ It comes as some surprise that platelets, which are devoid of genomic DNA, are equipped with and regulated by microRNAs. Despite lacking a nucleus, platelets contain messenger RNA (mRNA) splicing machinery and are able to translate mRNA into proteins.⁵ However, the in vivo contribution of de novo synthesized platelet proteins is incompletely understood. It is also currently unclear whether microRNAs have any function in platelets or if they act solely on the megakaryocyte level.

Several in vitro studies provided solid evidence for a role of microRNAs in megakaryocyte development and platelet production. These modulations could subsequently affect platelet activation and



Regulation of Fc γ RIIA signaling by TULA-2 and miR-148a. Binding of immune complexes to platelet Fc γ RIIA receptor leads to platelet activation and subsequent thrombotic complications resulting in heparin- and immune-induced thrombocytopenia. Platelet TULA-2 negatively regulates platelet activation downstream of Fc γ RIIA by diminishing spleen tyrosine kinase (Syk) phosphorylation (P). Intraplatelet TULA-2 levels therefore mediate Fc γ RIIA-induced platelet activation and thereby determine platelet responsiveness to heparin- or immune-mediated antigen-antibody complexes. In turn, intraplatelet TULA-2 is negatively regulated by microRNA miR-148a. Treatment of mice with anti-miR-148a results in diminished thrombotic complications in vivo.

reactivity. Megakaryocytes equip platelets with RNA and microRNA, thereby allowing platelets to mirror the condition of their progenitors. Platelets further take up various RNAs from cells they encounter within the circulation. Platelets also transfer mRNA and microRNAs from one cell to another via release of microvesicles, which allows platelets to communicate with a variety of different cell types, thereby modulating target cell effector functions in various diseases.⁶ Emerging lines of evidence indicate that platelet microRNAs are biologically and clinically relevant regulators of protein translocation and may serve as biomarkers for thrombotic disease and platelet reactivity, with possible therapeutic applications in the future.⁴

Zhang et al show for the first time that anti-microRNA therapy can inhibit platelet function in vivo. In an animal model, the authors demonstrate that anti-miR-148a enhances TULA-2 expression in bone marrow cells. This results in a strong reduction of microvessel occlusion and thrombotic events after use of an immune complex-induced in vivo platelet stimulation model. This model mimics the pathological situation of HIT in high responders and demonstrates the importance of TULA-2 function in FcγRIIA-mediated platelet signaling.

Whether the in vivo effects observed by anti-miR-148a are solely the result of FcγRIIA signaling in platelets or whether other cells are also involved remains to be determined. Recently, it was also shown that FcγRIIA signaling in monocytes contributes to thrombotic complications. Monocyte FcγRIIA signaling induces tissue factor production and subsequent thrombin generation in an Syk-dependent fashion.⁷ Therefore, anti-miR-148a treatment might also have beneficial antithrombotic effects on cell types other than platelets.

The study by Zhou et al brings us one step closer to understanding the underlying mechanism of inter-individual differences in response to FcγRIIA-mediated signaling in platelets. TULA-2 and miR-148a could potentially serve as predictive markers to identify patients at risk of HIT. However, the utility of anti-miR-148a as a therapeutic target is doubtful, because miR-148a has many crucial functions in other cell types. It was previously reported that miR-148a is a tumor-suppressive microRNA that potently induces apoptosis of tumor cells.⁸ Therefore

miR-148a itself has been suggested as a therapeutic target, which, given the effects of this microRNA on platelet activation and subsequent thrombotic complications demonstrated by Zhou et al, should be reconsidered.

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

REFERENCES

1. Zhou Y, Abraham S, Andre P, et al. Anti-miR-148a regulates platelet FcγRIIA signaling and decreases thrombosis in vivo in mice. *Blood*. 2015;126(26):2871-2881.
2. Greinacher A. Heparin-induced thrombocytopenia. *N Engl J Med*. 2015;373(3):252-261.
3. Tsygankov AY. TULA-family proteins: a new class of cellular regulators. *J Cell Physiol*. 2013;228(1):43-49.

4. Edelstein LC, McKenzie SE, Shaw C, Holinstat MA, Kunapuli SP, Bray PF. MicroRNAs in platelet production and activation. *J Thromb Haemost*. 2013;11(Suppl 1):340-350.
5. Denis MM, Tolley ND, Bunting M, et al. Escaping the nuclear confines: signal-dependent pre-mRNA splicing in anucleate platelets. *Cell*. 2005;122(3):379-391.
6. Rondina MT, Weyrich AS. Regulation of the genetic code in megakaryocytes and platelets. *J Thromb Haemost*. 2015;13(Suppl 1):S26-S32.
7. Tutwiler V, Madeeva D, Ahn HS, et al. Platelet transactivation by monocytes promotes the prothrombotic state in heparin-induced thrombocytopenia [published online ahead of print October 20, 2015]. *Blood*. doi:10.1182/blood-2013-11-539262.
8. Zhang H, Li Y, Huang Q, et al. MiR-148a promotes apoptosis by targeting Bcl-2 in colorectal cancer. *Cell Death Differ*. 2011;18(11):1702-1710.

DOI 10.1182/blood-2015-11-679860

© 2015 by The American Society of Hematology

● ● ● TRANSPLANTATION

Comment on Hiwarkar et al, page 2882

Cord blood T cells are “completely different”

Juliet Barker and Alan Hanash MEMORIAL SLOAN-KETTERING CANCER CENTER

According to some, one of Britain’s significant contributions to the modern world is the comedy of the Monty Python Flying Circus. One of their films employed the memorable catchphrase, “*And now for something completely different...*” Now, decades later, in this issue of *Blood*, the work of Hiwarkar et al represents another notable English export.¹ This group of investigators has begun to dissect the differences in graft-versus-tumor effects of adult donor peripheral blood (PB) and cord blood (CB) T cells, and the findings are quite remarkable.

For many years, CB transplantation (CBT) clinicians have observed and reported upon the unique nature of CBT biology based on observations on the transplant floor and in the clinic, as well as findings from single-center and registry studies of adult and pediatric CBT. The first remarkable feature of CBT is that the incidence of severe acute graft-versus-host disease (GVHD) is much lower than would be expected based on the high degree of donor-recipient human leukocyte antigen (HLA) mismatch. For example, the median 8 HLA-allele match of CB units transplanted at Memorial Sloan-Kettering Cancer Center (MSKCC) is 5/8 with grafts as mismatched as 3-4/8 frequently being administered to adult patients. And yet, the incidence of severe acute GVHD in CBT recipients is no greater than

that of HLA-matched adult donor allografts in many series. Recent MSKCC and University of Minnesota analyses have shown a day 100 grade 2 to 4 acute GVHD incidence of <15% in double-unit CB recipients who were transplanted with adequately dosed mycophenolate mofetil.^{2,3} The manifestations of chronic GVHD in CBT recipients are also different from that of adult donor PB allograft recipients with severe chronic GVHD being quite uncommon.⁴

It would be logical to assume that a less than expected GVHD risk would be associated with an increased incidence of relapse. In fact, the opposite has been observed, with multiple reports demonstrating that T-replete CBT with either single- or double-unit grafts is associated with a robust graft-versus-leukemia