Received: 26 May 2021 | Revised: 31 May 2021 | Accepted: 5 June 2021

DOI: 10.1002/rth2.12564

rpth research & practice in thrombosis & haemostasis

COMMENTARY

A tail of two ITAMs: GPVI/FcRγ and FcγRIIa's role in platelet activation and thrombus stability

Keith B. Neeves PhD

Department of Bioengineering, Department of Pediatrics, Section of Hematology, Oncology, and Bone Marrow Transplant, Hemophilia and Thrombosis Center, University of Colorado Denver, Anschutz Medical Campus, Aurora, CO, USA

Correspondence
Keith B. Neeves, University of Colorado Anschutz Medical Campus, 12800 East 19th Avenue, Mail Stop 8302, Aurora, CO 80045.
Email: keith.neeves@cuanschutz.edu

Handling Editor: Dr Alisa Wolberg.

Platelets are the ultimate cellular shapeshifters; they are hole pluggers, soldiers against invaders, wound healers, and cargo transporters. One of their more interesting tricks is that they have adapted immune-type receptors to service their role in hemostasis and vascular integrity. Receptors with an immunoreceptor tyrosine-based activation motif (ITAM) that contain two YxxL/I sequences on their cytoplasmic tail are important in supporting the signaling and activation of the integrins that are essential to platelet function. ITAMs and integrins both signal through Src family kinases, with subsequent activation of spleen tyrosine kinase and phospholipase Cγ2, leading to increased cytosolic calcium (Figure 1).[1,2] This shared signal transduction pathway allows ITAMs to enhance integrin outside-in signaling and sustain integrin activation.

Platelets have two receptors with the ITAM sequence, Fc receptor γ chain (FcRγ) and FcγRIIa.[3] FcRγ is noncovalently associated with glycoprotein VI (GPVI) and is essential for signaling downstream of GPVI binding to its ligands. GPVI contains an IgG-like domain that is homologous to immune receptors but is expressed only on platelets and megakaryocytes. It has a proline-rich domain that allows it to signal much faster than a typical immune receptor.[4] FcγRIIa is a low-affinity IgG receptor, which is expressed on human but not mouse platelets.[5] It binds to immune complexes leading to the spectrum of platelet activation responses including integrin activation, granule secretion, and phosphatidylserine exposure. FcγRIIa is the target of several autoimmune disorders involving antigen-antibody clustering, for example, heparin-induced thrombocytopenia. It also may support hemostasis by amplifying outside-in signaling of the integrin $\alpha_{IIb}\beta_3$ in the absence of binding to its own ligands, although the precise molecular mechanism of this interaction is unknown (Figure 1).[6,7] In this issue of *Research and Practice in Thrombosis and Haemostasis*, Ahmed and colleagues[8] examine the relative roles of GPVI/FcRγ and FcγRIIa on platelet activation on fibrinogen and thrombus formation and stability formed under flow on collagen.

The number of GPVI ligands has expanded beyond its original discovery as a collagen signaling receptor, and includes laminin, fibronectin, vitronectin, and most recently fibrinogen.[9,10] This interaction with fibrinogen is particularly important in reexamining the mechanisms of platelet aggregation. While $\alpha_{IIb}\beta_3$ is essential for fibrinogen-mediated platelet aggregation, extending their previous studies,[11] Ahmed and colleagues show that GPVI/FcRγ plays a supporting role in platelet activation on fibrinogen and platelet aggregation in thrombi formed under flow. Human platelets treated with a GPVI blocking antibody (IG5) show a significant inhibition of spreading on fibrinogen. When IG5 is perfused over preformed platelet aggregates, they disaggregate, leaving behind mostly those platelets directly bound to collagen. These observations suggest GPVI/FcRγ signaling is important for maintaining $\alpha_{IIb}\beta_3$ activation within a platelet aggregate. Wild type mouse platelets do not readily spread on fibrinogen without external stimuli, but mouse platelets expressing human GPVI were shown to spread on fibrinogen with no external stimulus. These data support a mechanism whereby GPVI/FcRγ sustains $\alpha_{IIb}\beta_3$ activation.

Inhibition of FcγRIIa shows more modest results compared to inhibition of GPVI/FcRγ in platelet spreading and disaggregation assays reported by Ahmed and colleagues. Human platelets treated with a FcγRIIa blocking antibody (IV.3) spread to a similar

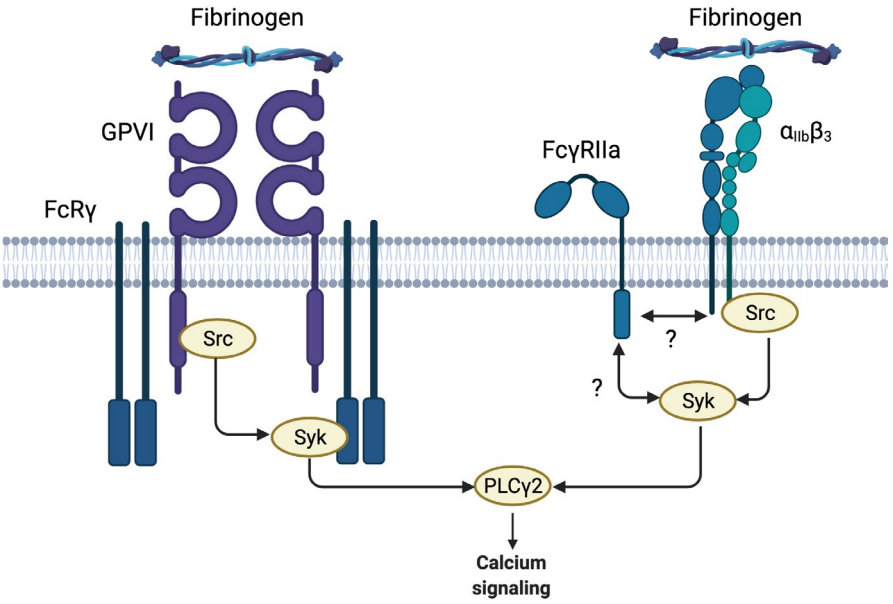

FIGURE 1 Potential signaling pathways for fibrinogen-mediated platelet activation. Fibrinogen can bind to glycoprotein VI (GPVI) and the integrin αIIbβ3. Fibrinogen binding results in phosphorylation of Src family kinases (Src) and spleen tyrosine kinase (Syk) associated with FcRγ and FcγRIIa. Integrins can also signal through Syk in the absence of ITAMs. Syk activated phospholipase Cγ2 (PLCγ2), leading to calcium release from intracellular stores. Created with BioRender.com

extent as a Fab fragment control. Treatment of preformed platelet aggregates with IV.3 did not result in disaggregation. Mouse platelets expressing human FcγRIIa do appear to spread slightly more than wild-type platelets, in agreement with previous results,[7] although the trend is not significant, potentially due to a low number of replicates. Some of these results challenge the in vitro studies by Newman and colleagues.[6,7] However, they also report a pronounced phenotype in two in vivo models of vascular injury in mice expressing human FcγRIIa on their platelets.[7] Moreover, ITAMs are known to serve a supporting role in integrin outside-in signaling in several other cell types.[12] Taken together, these conflicting data suggest that further research is needed to refine the role of FcγRIIa in platelet function outside of its contribution to thromboinflammation.[5]

There are several implications of these observations with respect to the contribution of platelet ITAMs on thrombus formation. First, for the platelet researcher, results from two common functional assays—spreading on fibrinogen and clot contraction—may need to be reinterpreted as not solely a means to test $\alpha_{IIb}\beta_3$ outside-in signaling given GPVI's ability to signal through fibrin(o-gen). Second, the expression levels of these receptors may influence their relative importance in supporting fibrinogen-dependent platelet aggregation. GPVI and FcγRIIa are expressed at similar copy numbers (1000s/platelet) and their expression is known to vary significantly in humans.[13,14] For example, there are two common alleles of GPVI, with one of them results in lower expression and thus reduced thrombus size under flow.[15] It is possible that lower levels of one ITAM could be compensated with higher levels of another ITAM. Likely, the local milieu of cytokines, chemokines, and metabolites and the nature of the vascular injury will determine the relative roles of these ITAMs. Finally, targeting ITAM receptors as an antithrombotic target could lead to less of a bleeding risk than direct inhibition of $\alpha_{IIb}\beta_3$. We may know the answer soon, as several therapeutic strategies for targeting GPVI have been developed and clinical trials are under way.[16]

ACKNOWLEDGEMENTS

The author would like to thank Peter Newman and Pierre Mangin for helpful conversations and feedback.

RELATIONSHIP DISCLOSURE

The author declares no conflicts of interest.

AUTHOR CONTRIBUTIONS

K.N. wrote the manuscript.

ORCID

Keith B. Neeves https://orcid.org/0000-0001-7546-4588

TWITTER

Keith B. Neeves @neeveslab

REFERENCES

1. Senis YA, Nagy Z, Mori J, Lane S, Lane P. Platelet Src family kinases: a tale of reversible phosphorylation. *Res Pract Thromb Haemost*. 2021;5:376-389.
2. Durrant TN, van den Bosch MT, Hers I. Integrin αIIbβ3 outside-in signaling. *Blood*. 2017;130:1607-1619.
3. Lee RH, Bergmeier W. Platelet immunoreceptor tyrosine-based activation motif (ITAM) and hemITAM signaling and vascular integrity in inflammation and development. *J Thromb Haemost*. 2016;14:645-654.
4. Schmaier AA, Zou Z, Kazlauskas A, et al. Molecular priming of Lyn by GPVI enables an immune receptor to adopt a hemostatic role. *Proc Natl Acad Sci*. 2009;106:21167-21172.
5. Patel P, Michael JV, Naik UP, McKenzie SE. Platelet FcγRIIA in immunity and thrombosis: adaptive immunothrombosis. *J Thromb Haemost*. 2021;19:1149-1160.
6. Boylan B, Gao C, Rathore V, Gill JC, Newman DK, Newman PJ. Identification of FcgammaRIIa as the ITAM-bearing receptor mediating alphaIIbbeta3 outside-in integrin signaling in human platelets. *Blood*. 2008;112:2780-2786.
7. Zhi H, Rauova L, Hayes V, et al. Cooperative integrin/ITAM signaling in platelets enhances thrombus formation in vitro and in vivo. *Blood*. 2013;121:1858-1867.

8. Ahmed MU, Receveur N, Janus-Bell E, et al. Respective roles of GPVI and FcγRIIA in the regulation of αIIbβ3-mediated platelet activation to fibrinogen, thrombus build-up and stability. *Res Pract Thromb Haemos*. 2021;5:e12551. https://doi.org/10.1002/rth2.12551
9. Rayes J, Watson SP, Nieswandt B. Functional significance of the platelet immune receptors GPVI and CLEC-2. *J Clin Invest*. 2019;129:12-23.
10. Xu RG, Gauer JS, Baker SR, et al. GPVI (glycoprotein VI) interaction with fibrinogen is mediated by avidity and the fibrinogen αC-region. *Arterioscler Thromb Vasc Biol*. 2021;41:1092-1104.
11. Mangin PH, Onselaer MB, Receveur N, et al. Immobilized fibrinogen activates human platelets through glycoprotein VI. *Haematologica*. 2018;103:898-907.
12. Mócsai A, Abram CL, Jakus Z, Hu Y, Lanier LL, Lowell CA. Integrin signaling in neutrophils and macrophages uses adaptors containing immunoreceptor tyrosine-based activation motifs. *Nat Immunol*. 2006;7:1326-1333.
13. Karas SP, Rosse WF, Kurlander RJ. Characterization of the IgG-Fc receptor on human platelets. *Blood*. 1982;60:1277-1282.
14. Joutsi-Korhonen L. The low-frequency allele of the platelet collagen signaling receptor glycoprotein VI is associated with reduced functional responses and expression. *Blood*. 2003;101:4372-4379.
15. Neeves KB, Onasoga AA, Hansen RR, et al. Sources of variability in platelet accumulation on type 1 fibrillar collagen in microfluidic flow assays. *PLoS One*. 2013;8:e54680.
16. Borst O, Gawaz M. Glycoprotein VI - novel target in antiplatelet medication. *Pharmacol Ther*. 2021;217:107630.

How to cite this article: Neeves KB. A tail of two ITAMs: GPVI/FcRγ and FcγRIIa's role in platelet activation and thrombus stability. *Res Pract Thromb Haemost*. 2021;5:e12564. https://doi.org/10.1002/rth2.12564