

COMMENTARY

A tail of two ITAMs: GPVI/FcR γ and Fc γ RIIa's role in platelet activation and thrombus stability

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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 C γ 2, 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 (FcR γ) and Fc γ RIIa.³ FcR γ 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 Fc γ RIIa 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. Fc γ RIIa 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 colleagues⁸ examine the relative roles of GPVI/FcR γ and Fc γ RIIa 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/FcR γ 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/FcR γ 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/FcR γ sustains $\alpha_{IIb}\beta_3$ activation.

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

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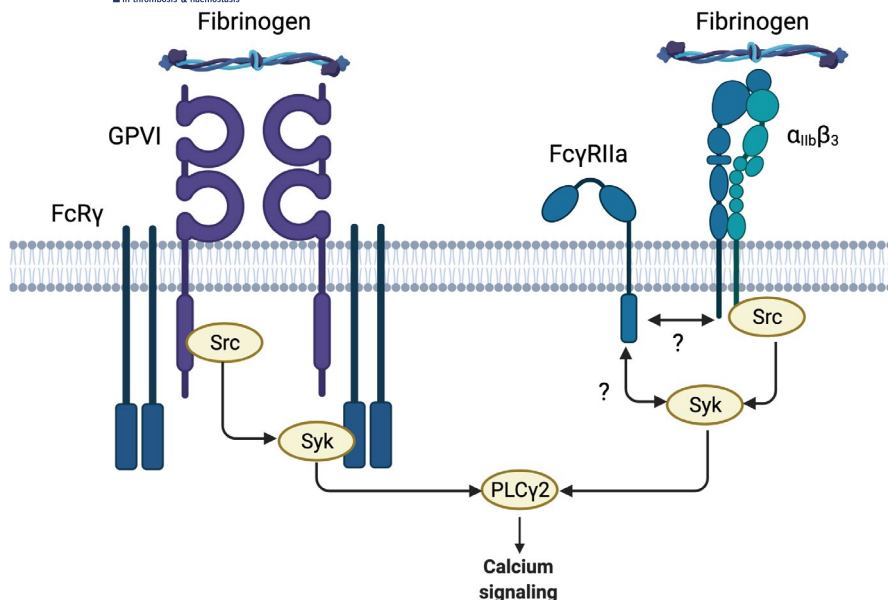


FIGURE 1 Potential signaling pathways for fibrinogen-mediated platelet activation. Fibrinogen can bind to glycoprotein VI (GPVI) and the integrin $\alpha\text{IIb}\beta_3$. Fibrinogen binding results in phosphorylation of Src family kinases (Src) and spleen tyrosine kinase (Syk) associated with FcR γ and Fc γ RIIa. Integrins can also signal through Syk in the absence of ITAMs. Syk activated phospholipase C γ 2 (PLC γ 2), 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 Fc γ RIIa 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 Fc γ RIIa 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 Fc γ RIIa 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 $\alpha\text{IIb}\beta_3$ 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 Fc γ RIIa 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 $\alpha\text{IIb}\beta_3$. 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.

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