SCHOOL OF MEDICINE
Biochemistry and Biophysics

SCHOOL OF MEDICINE
Cell Biology and Physiology

SCHOOL OF MEDICINE
Blood Research Center

Investigating a potential role for integrin activation and aggregation in immune thrombocytopenia

David Rocco[1], Robert H. Lee[2], Wolfgang Bergmeier[1,2]

University of North Carolina at Chapel Hill, [1]Department of Cell Biology and Physiology, [2]Department of Biochemistry and Biophysics

Background

Platelets are critical to hemostasis and wound healing through platelet plug formation via integrin activation and platelet aggregation. Engagement of platelet surface receptors induces various intracellular signals, which converge on activation of the small GTPase, Rap1. Activation of Rap1, in coordination with the cytoskeletal adapter protein, talin1, mediate activation of the integrin αIIbβ3. Platelets are first localized to the wound through the physical interaction between von Willebrand Factor (VWF) and the platelet receptor complex GPIb-IX. Interestingly, the alpha subunit of GPIb-IX (GPIbα) is a target in immune thrombocytopenia (ITP), where engagement of autoantibodies cause rapid platelet clearance from circulation. Studies suggest that the clearance mechanism does not proceed through Fc-mediated phagocytosis but is instead due to platelet activation. However, if and how Rap1 contributes to this process has not been well studied. To address this question, we use a polyclonal mixture of antibodies against GPIbα in combination with genetic knockout mice lacking various regulators of αIIbβ3 activation.

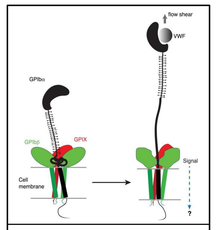


Figure 1: (a) Illustration showing mechanotransduction via GPIb-IX in response to ligand binding under shear force[1].

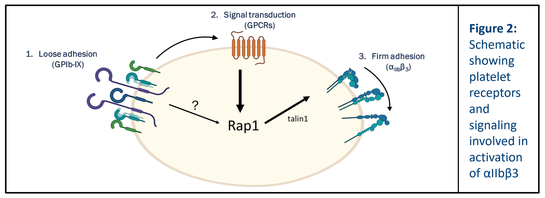
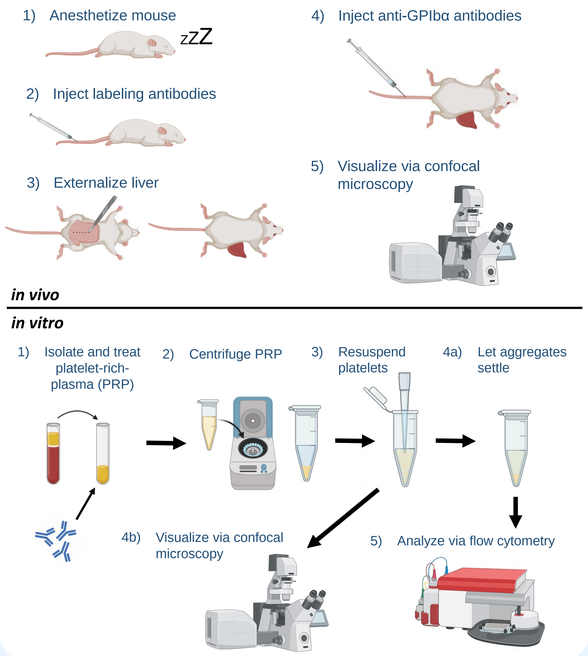


Figure 2: Schematic showing platelet receptors and signaling involved in activation of αIIbβ3

Methods



Results

Anti-GPIbα antibodies injected into mice causes platelet depletion and clearance by liver macrophages

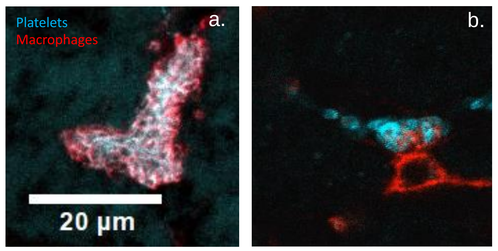


Figure 3: Confocal microscopy of fixed liver section (a) and still image from real-time intravital imaging (b) following platelet clearance induced by anti-GPIbα antibodies.

Antibodies against GPIbα cause cohesion of quiescent platelets

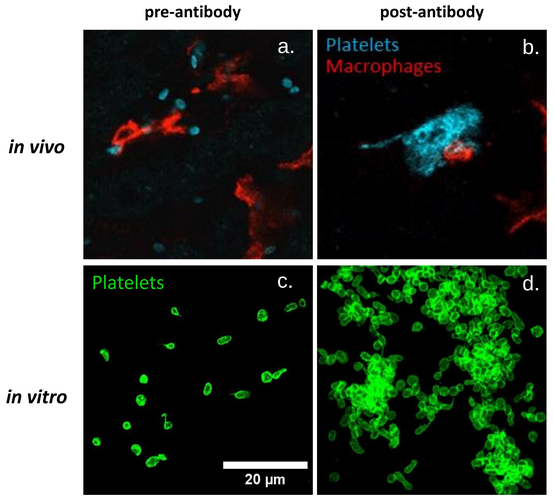


Figure 4: Stills from confocal intravital imaging *in vivo* (a,b) and fixed platelets *in vitro* before (a,c) and after (b,d) administration of anti-GPIbα antibodies.

Loss of GPIbα inhibits platelet response to anti-GPIbα antibodies

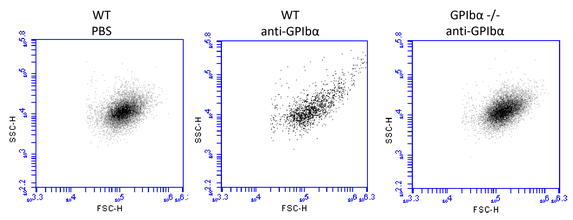


Figure 5: Platelet resuspension assay of platelets from wildtype or mutant mice lacking GPIbα.

Results (continued)

Platelet cohesion induced by anti-GPIbα antibodies is not dependent on integrin activation

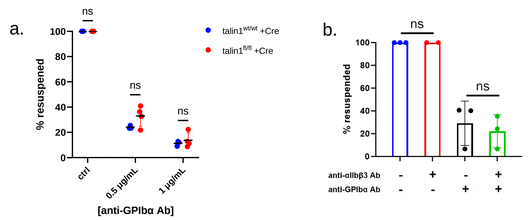


Figure 6: (a) Platelet resuspension assay of platelets from transgenic mice lacking talin1 and control mice. (b) Platelet resuspension assay of wildtype platelets centrifuged with anti-GPIb antibodies.

Loss of Rap1 reduces platelet cohesion and causes weak Rap1 activation in response to anti-GPIbα antibodies

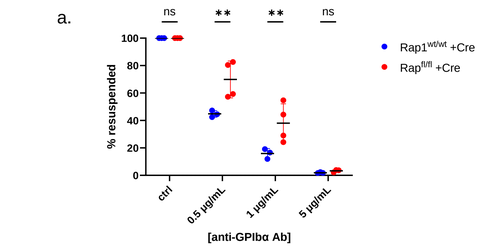


Figure 7: (a) Quantification of platelets from Rap1-null and control mice were subjected to platelet centrifugation and resuspension. (b) Quantification of platelet size from mice lacking Rap1 or control mice[2]. (c) Analysis of Rap1 activation following administration of anti-GPIbα antibodies.

Loss of Rap1 Regulators does not affect platelet adhesion induced by anti-GPIbα antibodies

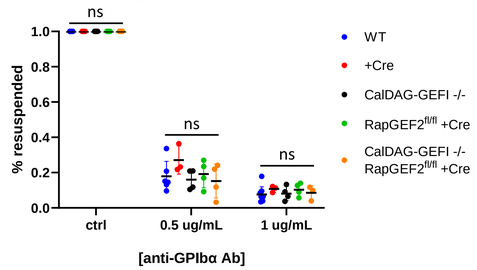


Figure 8: Platelet resuspension assay with mutant mice lacking the major (CalDAG-GEFI) and/or minor (RapGEF2) positive regulators of Rap1 with respective controls.

Summary and Conclusions

- GPIb-IX is important for hemostasis, especially under high shear conditions
- GPIbα is a major target of antibodies in immune thrombocytopenia
- Antibodies against GPIbα antibodies induce platelet cohesion *in vivo*, and *in vitro* with addition of centrifugal (mechanical) force
- Platelet cohesion is not mediated by activation of αIIbβ3
- Antibodies against GPIbα cause weak Rap1 activation, and reduced platelet cohesion at low concentrations
- Mice lacking primary and/or secondary positive regulators of Rap1 do not influence anti-GPIBα-mediated platelet cohesion

Conclusion: Administration of antibodies against GPIbα causes platelet cohesion both *in vivo* and *in vitro*, providing a model to study platelet clearance in GPIb-IX-mediated ITP. Though this process occurs independently of αIIbβ3, loss of Rap1 has a mild effect on platelet cohesion. However, absence of positive Rap1 regulators has no effect. Further investigation is needed to determine if reduction in platelet cohesion in Rap1-null mice is due to signaling and/or structural defects.

Future Directions

- Determine if defect in Rap1 mice is due to diminished intracellular signaling or platelet size/receptor avidity
- Visualize platelet clearance during and after in select mice *in vivo* to analyze change in kinetics, extent of clearance, platelet/agglomerate morphology, and phagocytosis
- Examine interaction between Rap1 and other small GTPases involved in cytoskeletal rearrangements
- Explore αIIbβ3-independent mechanisms of platelet cohesion
- Investigate how macrophages recognize and phagocytose platelet agglomerates and if Rap1 contributes to this process

Acknowledgements

Bergmeier Lab

UNC Blood Research Center

UNC Integrative Vascular Biology Program (2T32HL069768-21A1)

National Heart, Lung, and Blood Institute

bio RENDER

References

1. Zhang W, Deng W, Zhou L, et al. Identification of a juxtamembrane mechanosensitive domain in the platelet mechanosensor glycoprotein Ib-IX complex. Blood. 2015;125(3):562-569.
2. Stefanini L, Lee RH, Paul DS, et al. Functional redundancy between RAP1 isoforms in murine platelet production and function. Blood. 2018;132(18):1951-1962