

Platelet protein GPIb α plays an important role in regulating platelet-mediated production of thrombopoietin by the liver

What is this research about?

Platelets are tiny cell fragments that circulate at the periphery of the blood flow near the blood vessel wall. If they encounter damage, they become “activated”, changing shape and forming a clot. Platelets are essential for wound repair and to stop blood loss after injury. If a person has low platelet numbers or their platelets are not functioning well, they are at risk of bleeding.

Platelets lack a nucleus, and only live for about 10 days. They are produced by bigger cells in the bone marrow called megakaryocytes. Megakaryocytes release platelets into the blood stream continuously to ensure there are adequate numbers of platelets in circulation. On average, an adult produces about 100 billion platelets every day.

Production of platelets from megakaryocytes is regulated by a hormone called thrombopoietin (TPO), which is mostly produced by the liver. TPO circulates in the blood and binds to a receptor on the surface of platelets, called Mpl. Once bound, TPO is taken into the platelet and destroyed. It is thought that in this way, TPO's control of platelet production can be fine-tuned in response to platelet counts. When platelet counts are high, there is less TPO available to stimulate platelet production from megakaryocytes. Conversely, if platelet counts drop, less TPO will be bound to platelets and destroyed, more TPO will be available, and megakaryocytes will produce more platelets. This inverse correlation between TPO levels circulating in the blood and platelet/megakaryocyte mass is thought to be one of the major ways platelet production is regulated.

In this study, the researchers examined a platelet surface protein called GPIb α . This is part of a complex of proteins that is important in platelet adhesion and plug formation. People lacking GPIb α have a rare bleeding disorder called Bernard Soulier Syndrome, which is characterized by low platelet counts and abnormally large platelets. In mice lacking GPIb α , the researchers unexpectedly observed two to three times lower levels of TPO than in normal mice, leading them to hypothesize that GPIb α might affect TPO levels.

What did the researchers do?

The researchers wished to better understand how steady-state levels of TPO and platelets in the circulation are maintained. They investigated TPO in mice lacking GPIb α (GPIb α “knockouts”) and in blood samples from people who lack GPIb α . To study TPO in mouse platelets lacking GPIb α , they measured TPO levels in the liver and blood of these mice, and measured the clearance of TPO from the circulation by platelets. They also measured TPO RNA levels in the liver, which indicates how much TPO the liver is making. They transfused mice lacking GPIb α with normal mouse platelets to see whether having normal platelets would improve TPO production in the liver. Proteins on the surface of platelets are covered with sialic acid, a natural sugar, that helps protect the platelets from destruction. These sugar residues can play an important role in platelet function, and the researchers tested whether removing them from platelets impacted TPO production. They conducted experiments to see how well platelets lacking GPIb α bind to liver cells. Patients with immune thrombocytopenia, an autoimmune bleeding disorder, may develop antibodies against GPIb α , so to help understand any potential clinical implications, they also studied the effect of antibodies against GPIb α .

In brief...

Platelets, through GPIb α , contribute to the steady-state production of thrombopoietin by the liver. This has important implications in bleeding diseases such as Bernard Soulier Syndrome and immune-mediated thrombocytopenias.

What did the researchers find?

- ◆ In both mice and humans, lacking platelet GPIIb/IIIa causes less circulating TPO.
- ◆ Although mice lacking GPIIb/IIIa have larger platelets, there was no difference in amount of the Mpl receptor, or in the platelets' ability to clear TPO. Therefore, the lower TPO levels seen in mice lacking GPIIb/IIIa is not due to more clearance of TPO by platelets.
- ◆ Lower TPO levels in mice lacking GPIIb/IIIa is due to impaired production of TPO by the liver. The livers of mice lacking GPIIb/IIIa had about half the amount of TPO RNA compared to normal mice, indicating that their livers produce less TPO. Transfusing mice lacking GPIIb/IIIa with normal platelets could "rescue" this by increasing levels of circulating TPO and levels of TPO RNA in the liver.
- ◆ Platelets lacking GPIIb/IIIa do not bind well to liver cells, and do not stimulate the liver to produce TPO. Removing sugar residues from platelets of mice lacking GPIIb/IIIa did not improve TPO production.
- ◆ The extracellular domain of GPIIb/IIIa – the portion of the protein that extends out from the surface of the platelet – is necessary and sufficient for TPO production by the liver.
- ◆ Treating normal platelets with antibodies against GPIIb/IIIa blocks TPO production by the liver.

How can you use this research?

How TPO levels are regulated has been widely debated. This study identifies a previously unknown way to regulate circulating TPO levels and shows that GPIIb/IIIa is the essential link between the platelet and the liver cell in stimulating the liver to produce TPO. When GPIIb/IIIa is lacking, TPO production is impaired. Impaired TPO production was specific to the liver and was not seen in other organs that can produce TPO. This study suggests that platelets, through GPIIb/IIIa, contribute significantly to the steady-state production of TPO from the liver, advancing the fundamental understanding of platelet-mediated production of TPO.

The findings have potential implications in disease conditions. For example, patients who have an immune reaction to platelets produce antibodies against platelets, leading to platelet destruction, low platelet counts, and bleeding that may require platelet transfusions. In some patients, the anti-platelet antibodies target GPIIb/IIIa. This study shows that antibodies against GPIIb/IIIa could mimic the effects of GPIIb/IIIa deficiency and block production of TPO by the liver. This may explain unexpectedly low TPO levels observed in some of these patients, and help inform appropriate treatment choices, although further investigation is required.

About the research team: This study was led by **Heyu Ni**, a Canadian Blood Services scientist and a professor in the departments of medicine, laboratory medicine and pathobiology, and physiology at the University of Toronto. Dr. Ni is the platform director for hematology, cancer, and immunologic diseases at St. Michael's Hospital. The research team included members of Dr. Ni's laboratory (Miao Xu, June Li, Miguel Antonio Dias Neves, Guangheng Zhu, Naadiya Carrim, Ruoying Yu), Canadian Blood Services colleagues (**Donald R. Branch** and **Alan Lazarus**), and collaborators from Toronto (Sahil Gupta, John Marshall, Ori Rotstein, John Freedman), China (Jun Peng, Ming Hou), Japan (Shinji Kunishima), and the United States (Jerry Ware, Zaverio M. Ruggeri).

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[1] Xu M, Li J, Neves MAD, *et al.* GPIIb/IIIa is required for platelet-mediated hepatic thrombopoietin generation. *Blood* 2018; DOI 10.1182/blood-2017-12-820779.

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