Is the mysterious platelet receptor GPV an unsuspected major target for platelet autoantibodies?

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To confirm an origin of immune thrombocytopenia (ITP), serum autoantibodies are routinely tested for by enzyme-linked immunosorbent assay (ELISA), or monoclonal antibody immobilization of platelet antigens (MAIPA), with the integrin alpha IIb-beta 3 (αIIbβ3) and glycoprotein (GP)Ib-IX receptors on platelets as major targets.[1] A range of commercially available kits also allows the detection of human antibodies to these and other platelet targets. In spite of this, confirmation of an immune process for an acquired thrombocytopenia is often difficult to establish, as many potential antigenic targets occur on less well-characterized or minor surface components of platelets that are not tested for. Over the past 20 years, familial thrombocytopenia, often linked with increased platelet size, has no longer been systematically considered as ITP.[2] The recognition of a possible genetic origin, even in the absence of a family history, has been facilitated by the tremendous progress linked to the development of next generation sequencing procedures.[3] This revolution has reinforced the need for more positive criteria to diagnose ITP and to be able to elaborate the best strategy for their treatment.

In this issue of *Haematologica*, Vollenberg *et al.*[4] identify GPV, a little understood constituent of the GPIb-IX-V complex, as a frequent target for autoantibodies in ITP. Firstly, they developed methods to improve the detection of platelet-bound and free autoantibodies, and also to evaluate their pathological consequences, using a phagocytosis assay or a NOD/SCID mouse model. In particular, they applied surface plasmon resonance (SPR) technology using recombinant His-tagged GPV. With SPR, they were able to detect lower avidity autoantibodies in the sera of patients compared to indirect MAIPA. Using a direct MAIPA, they found platelet-bound anti-GPV autoantibodies either alone (2.9%) or more often associated with antibodies to GPIb-IX and/or to αIIbβ3 in 61.8% of 343 positive samples in a series of 1140 ITP patients. Free antibodies to GPV in the sera were found in 66.6% of the 45 patients found positive by indirect MAIPA, which is more frequent than those found for αIIbβ3. When sera from 222 patients positive for platelet-bound GPV were tested using SPR, 88 (39.6%) now tested positively, showing a higher specificity due to the ability of the SPR approach to detect lower avidity antibodies. It should be noted, however, that low avidity antibodies to αIIbβ3 and GPIb-IX were not evaluated in serum. Blocking with recombinant GPV confirmed the specificity for serum autoantibodies to GPV in MAIPA.

Vollenberg *et al.*[4] clearly demonstrated the role of anti-GPV autoantibodies in mediating platelet clearance. First, they showed that both high- and low-avidity anti-GPV were able to mediate platelet uptake in a phagocytosis assay using human macrophages. They then performed an *in vivo* experiment using a NOD/SCID mouse model with transfused human platelets. Their model was validated using a murine monoclonal antibody (MoAb) SW16 against human GPV injected at two concentrations that induced comparable clearance to a murine MoAb (SZ21) specific for αIIbβ3. Next, the IgG fraction of sera from patients containing exclusively anti-GPV autoantibodies of low- or high-avidity were administered. Interestingly, the human anti-GPV increased platelet clearance, although to a lower extent than was seen with SW16; results were similar for the high- or low-avidity IgG fractions and platelet survival was increased after adsorption of the sera with recombinant GPV. It should be noted, however, that the extent to which platelet GPV was saturated was not evaluated. In conclusion, as for classical autoantibodies in ITP, anti-GPV are able to cause thrombocytopenia.

The mechanism by which platelets are cleared for patients with anti-GPV antibodies is worthy of further investigation. Early biosynthesis and assembly of the GPIb-IX complex includes *N*-glycosylation of the individual subunits and extensive *O*-glycosylation of the extracellular mucin-like domain of GPIbα.[5] GPV (approx. 85 kDa) is non-covalently associated by way of transmembrane interactions with GPIbα and its association with GPIb-IX is required for full expression at the platelet surface.[6] Classically, the GPIb-IX-V complex is lacking or dysfunctional in patients with inherited biallelic Bernard-Soulier syndrome (BSS) where bleeding occurs through the adhesion defect caused by the absence of GPIbα, the platelet receptor for von Willebrand factor (VWF).[7] It has 50% expression in monoallelic forms of BSS linked to macrothrombocytopenia alone.[2,7] In contrast to the other subunits, the specific absence of GPV in mice (GPV$^{-/-}$) did not result in BSS, platelet and MK morphology were normal, and there was abundant GPIb-IX expression.[8] Classically, mutations in GPV do not give rise to human BSS.[7]

In ITP, pathways to remove platelets from the circulation include Fc-receptor-mediated clearance by mononuclear macrophages primarily, although not exclusively, located in the spleen.[9] Vollenberg *et al.*[4] clearly show that antibodies to GPV can bring about phagocytosis by macrophages. But are there other possible mechanisms? Platelet activation in platelets incubated with monoclonal antibodies (MoAbs) to GPIb (but not with antibodies to αIIbβ3) or in ITP patients with anti-GPIb autoantibodies can be followed by neuraminidase translocation to the platelet surface.[10] Removal of terminal sialic acid residues from the O-linked oligosaccharides of the mucin-like domain of GPIbα results in severe thrombocytopenia due

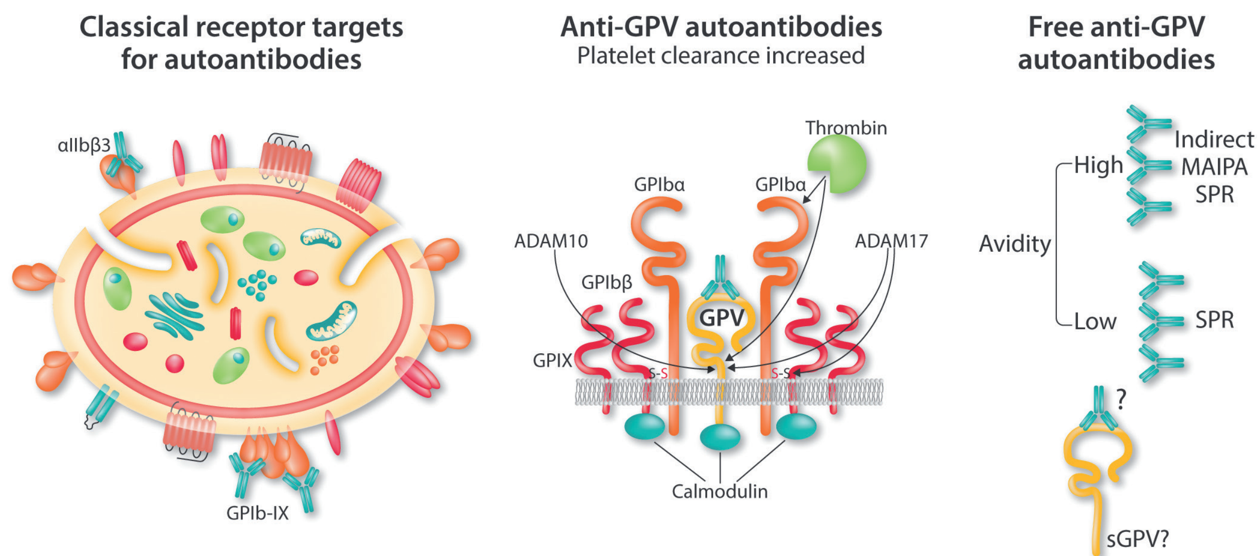

Figure 1. GPV, a major target for autoantibodies in immune thrombocytopenia (ITP). (Left) Representation of a platelet showing the two principal receptor targets for platelet autoantibodies. Vollenberg *et al.*,[4] while confirming the abundance of autoantibodies detected by direct MAIPA in a series of 343 positive samples from among 1140 tested ITP patients, also showed a significant presence of antibodies to GPV. (Middle) The GPIb-IX-V complex showing the presence of anti-GPV antibody and highlighting binding or cleavage sites for thrombin and metalloproteases ADAM10 and ADAM17. Vollenberg *et al.*[4] raise questions as to the consequences of bound autoantibodies on GPV structure, the cleavage of released soluble forms, and a potential desialylation of the subunits on platelet survival in ITP. (Right) The detection of both high avidity autoantibodies to GPV by MAIPA and additional low avidity antibodies by surface plasmon resonance (SPR) technology is intriguing, as both of these appear to contribute to the diagnosis of ITP.

to platelet clearance in the liver *via* αMβ2 on Kupffer cells or Ashwell-Morell receptors (AMR) on hepatocytes.[10] For GPV, loss of sialic acid occurs concomitantly to GPIbα after cold stored platelets are rewarmed, a process also followed by metalloprotease-induced shedding of these receptors.[11] It is now important to determine whether thrombocytopenia observed with anti-GPV antibodies results from a Fc-dependent clearance or from an Fc-independent mechanism implying liver receptors. Neuraminidases are present in platelet α-granules, but it is not known how they are translocated to the platelet surface or how they come into contact with sialic acid residues that are extended far from the surface. Is their action on the same platelet or after platelet-to-platelet contact? Not all anti-GPIb antibodies are capable of activating the GPIb-IX complex; significantly, some directed against the ligand binding domain induce the juxtamembrane mechanosensory domain (MSD) of GPIbα to be unfolded and Fc-independent platelet clearance by a mechanosensory mechanism.[12] It will be interesting to verify whether anti-GPV autoantibodies share some of these properties or will modify the sialic acid expression of GPIbα, or indeed of GPV itself. Heterogeneity in the response may be predicted, as infusion of rat MoAbs to murine GPV had no effect in a mouse model, whereas MoAbs to GPIbα resulted in thrombocytopenia and megakaryocyte abnormalities.[13] Along with the target on GPV, it is difficult to speculate as to the relevance of high and low avidity anti-GPV antibodies.

The GPV ectodomain contains: i) a cleavage site for thrombin, leading to loss of the bulk of the extracellular domain of GPV from the platelet surface and the generation of soluble GPV (sGPV); and ii) a second cleavage site for endogeneous metalloproteinases and, in particular, ADAM17, which is the major sheddase for GPV on the platelet surface (Figure 1).[14] Whether shedding of GPV is a contributor to or a consequence of immune clearance has still not been determined. The presence of elevated levels of sGPV in models of thrombosis suggests that it may be a marker of thrombotic activity.[15] The role of sGPV in the circulation remains unknown, as does the possible role of natural antibodies in clearing the soluble form.

So far, the physiological role and function, if any, of GPV remains elusive. $GPV^{-/-}$ mouse platelets are morphologically indistinguishable from wild-type platelets, but two reports show an increased platelet sensitivity to low doses of thrombin in the absence of GPV, suggesting an anti-thrombotic role for GPV.[9,16,17] In contrast, platelet GPV reportedly binds to collagen and participates in platelet adhesion and aggregation.[18] Mice lacking GPV have mildly reduced tail bleeding times and, depending on the severity of the injury, display slightly accelerated thrombus formation.[19] So the fascinating questions as to the potential value of GPV as an anti-thrombotic target and the possible modulating roles of sGPV remain. Are these observations sufficient to predict a modification of platelet function when anti-GPV antibodies are present? Only future studies will be able to provide an answer.

In their report, Vollenberg *et al.*[4] focus on the prevalence of auto anti-GPV antibodies and their contribution to platelet clearance. They clearly establish the importance of including this target in the diagnosis of ITP, and confirm the findings of previously published literature and of a recent short report by Porcelijn *et al.*[20] GPV should certainly form part of antibody testing kits, but more studies are required to evaluate their clinical rele-

vance. However, in spite of this, the manuscript of Vollenberg *et al.*[4] opens new fields of investigation concerning the evaluation of the mechanisms involved in immune thrombocytopenia and the bleeding tendency of the patients affected.

References

1. Kiefel V, Santoso S, Kaufmann E, Mueller-Eckhardt C. Autoantibodies against platelet glycoprotein Ib/IX: a frequent finding in autoimmune thrombocytopenic purpura. Br J Haematol. 1991;79(2):256-262.
2. Noris P, Balduini CL. Inherited thrombocytopenias in the era of personalized medicine. Haematologica. 2015;100(2):145-148.
3. Bastida JM, Lozano ML, Benito R, et al. Introducing high-throughput sequencing into mainstream genetic diagnostic practice in inherited platelet disorders. Haematologica. 2018;103(1):148-162.
4. Vollenberg, R, Jouni R, Norris PAA, et al. Glycoprotein V is a relevant immune target in patients with immune thrombocytopenia. Haematologica. 2019;104(6):1237-1243.
5. Ulsemer P, Strassel C, Baas MJ, et al. Biosynthesis and intracellular post-translational processing of normal and mutant platelet glycoprotein GPIb-IX. Biochem J. 2001;358(Pt 2):295-303.
6. Li CQ, Dong JF, Lanza F, Sanan DA, Sae-Tung G, Lopez JA. Expression of platelet glycoprotein (GP) V in heterologous cells and evidence for its association with GPIbalpha in forming a GPIb-IX-V complex on the cell surface. J Biol Chem. 1995;270(27):16302-16307.
7. Savoia A, Pastore A, DE Rocco D, et al. Clinical and genetic aspects of Bernard-Soulier syndrome: searching for genotype/phenotype correlations. Haematologica. 2011;96(3):417-423.
8. Poujol C, Ramakrishnan V, DeGuzman F, Nurden AT, Phillips DR, Nurden P. Ultrastructural analysis of megakaryocytes in GPV knockout mice. Thromb Haemost. 2000;84(2):312-318.
9. McMillan R. The pathogenesis of chronic immune thrombocytopenic purpura. Semin Hematol. 2007;44(4 Suppl 5):S3-S11.
10. Li J, van der Wal DE, Zhu G, et al. Desialylation is a mechanism of Fc-independent platelet clearance and a therapeutic target in immune thrombocytopenia. Nat Commun. 2015;6:7737.
11. Jansen AJ, Josefsson EC, Rumjantseva V, et al. Desialylation accelerates platelet clearance after refrigeration and initiates GPIbα metalloproteinase-mediated cleavage in mice. Blood. 2012;119(5):1263-1273.
12. Quach ME, Dragovich MA, Chen W, et al. Fc-independent immune thrombocytopenia via mechanomolecular signaling in platelets. Blood. 2018;131(7):787-796.
13. Poujol C, Bergmeier W, Nurden P, et al. Effect of infusing rat monoclonal antibodies to the murine GPIb-IX-V complex on platelet and megakaryocyte morphology in mice. Platelets. 2003;14(1):35-45.
14. Bender M, Stegner D, Nieswandt B. Model systems for platelet receptor shedding. Platelets. 2017;28(4):325-332.
15. Ravanat C, Freund M, Mangin P, et al. GPV is a marker of in vivo platelet activation--study in a rat thrombosis model. Thromb Haemost. 2000;83(2):327-333.
16. Kahn ML, Diacovo TG, Bainton DF, Lanza F, Trejo J, Coughlin SR. Glycoprotein V-deficient platelets have undiminished thrombin responsiveness and do not exhibit a Bernard-Soulier phenotype. Blood. 1999; 94(12):4112-4121.
17. Ramakrishnan V, Reeves PS, DeGuzman F, et al. Increased thrombin responsiveness in platelets from mice lacking glycoprotein V. Proc Natl Acad Sci USA 1999;96(23):13336-13341.
18. Moog S, Mangin P, Lenain N, et al. Platelet glycoprotein V binds to collagen and participates in platelet adhesion and aggregation. Blood. 2001;98(4):1038-1046.
19. Nonne C, Hechler B, Cazenave JP, et al. Reassessment of in vivo thrombus formation in glycoprotein V deficient mice backcrossed on a C57Bl/6 strain. J Thromb Haemost. 2008;6(1):210-212.
20. Porcelijn L, Huiskes E, Oldert G, et al. Detection of platelet autoantibodies to identify immune thrombocytopenia: state of the art. Br J Haematol. 2018;182(3):423-426.