Platelet adhesion, activation, and aggregation at sites of vascular injury are crucial for normal hemostasis but may also lead to occlusion of diseased vessels, resulting in myocardial infarction or ischemic stroke. The extracellular matrix protein collagen is the major and most thrombogenic component of the vessel wall, as it provides an adhesion substrate for platelets and directly activates the cells. This activation is mediated by the platelet-specific collagen receptor glycoprotein (GP) VI.

GPVI is a transmembrane type I receptor of the immunoglobulin superfamily that noncovalently associates with the immunoreceptor tyrosine-based activation motif containing Fc receptor γ-chain. On ligand-induced GPVI clustering, the Fc receptor γ-chain becomes tyrosine phosphorylated, initiating a series of tyrosine phosphorylation events that finally result in cellular activation. In vitro studies have shown that monomeric GPVI has virtually no affinity for fibrillar collagen, whereas a dimeric form of GPVI, comprising the extracellular domain of the receptor fused to human immunoglobulin Fc domain (GPVI-Fc), binds with high affinity to collagen and collagen-related peptides. Furthermore, the idea that collagen binding is mediated by GPVI dimers at the platelet surface was corroborated by crystallographic data, as well as by studies using synthetic peptides with differentially spaced GPVI binding motifs and studies using chemical cross-linking agents. However, despite these biochemical data, the valence of GPVI on resting platelets has not unequivocally been revealed, and the implication of GPVI dimerization in platelet activation is still a matter of debate.

In the current issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Loyau et al. report the generation of a novel monoclonal antibody (9E18), which in vitro strongly binds to dimeric human GPVI (GPVI-Fc), whereas it displays only very low affinity for the respective monomeric form (GPVI-His). Using this unique tool, they provide strong evidence that the majority of GPVI is expressed in a monomeric form on resting human platelets and that platelet stimulation by soluble agonists such as ADP or TRAP results in the formation of GPVI dimers at the platelet surface. Interestingly, the increased 9E18 binding correlated with P-selectin exposure, a marker of activation-dependent α-granule secretion, but it remained unclear whether granule release is a prerequisite for agonist-induced GPVI dimerization under these conditions.

At high shear flow rates, the initial capture of circulating platelets on the extracellular matrix is mediated by the interaction of GPIbα and collagen-bound von Willebrand factor slowing down the cells and favoring GPVI interaction with collagen. By applying high shear to platelet-rich plasma, as well as by adding von Willebrand factor to shear-stressed washed platelets, Loyau et al. demonstrate that platelet activation by GPIbα/von Willebrand factor interactions induces dimerization of surface GPVI and thereby primes the receptor for its interaction with collagen. These findings provide the first experimental evidence that GPVI, similar to integrin adhesion receptors, may have to become “inside-out activated” to efficiently bind its ligand and induce full cellular activation. If this holds true, GPVI dimerization may represent a novel checkpoint in the thrombotic cascade that enables fine-tuning of platelet activation and coagulant activity at sites of vascular injury (Figure).

In the second part of the study, the authors assessed the mechanism underlying activation-dependent GPVI dimerization in more detail. Interestingly, they found that GPVI dimerization is controlled by the intracellular concentration of cAMP but that neither Fc receptor γ-chain phosphorylation nor the activation of tyrosine-kinases downstream of GPVI is required for dimerization.

cAMP is known as a powerful inhibitor of platelet aggregation, and adenylate cyclase is inhibited by the Gi-coupled ADP receptor P2Y12 and activated by the G1-coupled prostacyclin receptor. Furthermore, endothelial cell-derived nitric oxide and prostacyclin inhibit platelet activation by increasing the intracellular cGMP concentration. Loyau et al. now provide evidence that this pathway also inhibits GPVI dimerization, thereby maintaining platelets in a hyporeactive state. A loss of this protective effect due to endothelial dysfunction, damage of the vessel wall, or high shear-induced GPIbα/von Willebrand factor interaction would then exert a priming effect, permitting a more rapid or more efficient interaction of platelets with collagen. Future studies will need to focus on the exact signaling mechanism underlying cAMP/cGMP-regulated GPVI dimerization.

In a third part of their study, the authors assessed the clinical relevance of their findings by measuring the binding of 9E18 and exposure of P-selectin in platelet-rich plasma from a cohort of patients with coronary artery disease treated with dual antiplatelet therapy (aspirin and clopidogrel). Indeed, they found a correlation of GPVI dimerization and P-selectin exposure in these patients, leading them to the proposal that dimeric GPVI levels could represent a new and early biological marker of platelet (pre)activation. Clearly, however, additional
studies will be required to prove the principal utility and sensitivity of a diagnostic test assessing GPVI dimerization in age- and sex-matched patients with other cardiovascular diseases. If these studies confirm and extend the initial observations reported here by Loyau et al., routine detection of GPVI dimers could become a valuable diagnostic tool.

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Disclosures
None.

References

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Sebastian Dütting and Bernhard Nieswandt

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