in the case of GPIb-IX, the platelet situation includes an additional subunit, GP V, often referred to as the GPIb-IX-V complex. Could the absence of GP V in the heterologous cell model alter the in vivo response to thrombin? It is known that GP V binds thrombin and that GP V-deficient mice have an altered thrombin response. Thus, the in vivo situation could be more complex. In addition, how faithfully does the Chinese hamster ovary cell recapitulate the signaling pathways of a circulating platelet? The same group has previously described a membrane permeable peptide of cytoplasmic GPIb sequence that elicits mutually dependent signals via the 14-3-3-zeta binding site within the cytoplasmic domain of platelet glycoprotein Ib-IX. However, there still could be subtle differences between heterologous cells and platelets impacting the major thrombin-centric conclusions in the current work. Yes, the heterologous cell model is a powerful and valuable experimental tool, but it is worth remembering that it is not a platelet. The future direction from this current work will likely be a more rigorous test of the cooperativity model in a platelet setting.

Going forward, could these data support the development of antagonists that selectively target the cooperative pathways of PARs and GPIb-IX? Certainly, this new work from Estevé et al’s laboratory leads us in that direction. Even more widespread interest would be generated if these antagonists were not only beneficial as anti-thrombotics, but also displayed anti-inflammatory and/or anti-cancer benefits. More studies will likely follow and the widespread availability of platelet-specific reagents and experimental approaches gives the work reported in this issue of Blood a high level of interest and importance.

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WF is a multimeric glycoprotein that plays a major role in hemostasis and thrombosis by promoting adhesion to platelet receptors and platelet-platelet interactions under high shear stress conditions. VWF is synthesized by vascular endothelial cells (ECs) and megakaryocytes in ultra-large (UL) molecular weight multimers that can be stored in Weibel-Palade bodies and platelet granules. After secretion, UL-VWF remains on the cell surface as very long strings that may interact actively with circulating platelets. UL-VWF in circulation is converted to the smaller and less thrombogenic forms of VWF by the metalloprotease ADAMTS13, which cleaves the Tyr1605-Met1606 bond of the VWF A2 domain. Quantitative VWF defects and/or qualitative abnormalities of VWF structure cause bleeding disorders such as inherited von Willebrand disease whereas increased levels of VWF and/or the persistence of the UL-VWF in circulation are responsible for thrombotic disorders such as thrombotic microangiopathies (TMAs) and other arterial thrombosis.

Platelet adhesion and aggregation at sites of vascular injury under shear stress conditions such as those found in microcirculation and in large arteries with lumen restrictions are dependent on the normal multimeric VWF. In blood flow, the VWF A1 domain mediates platelet adhesion by interacting with the glycoprotein (GP) V receptor. Platelets become activated following the initial tethering which allows their irreversible adhesion followed by binding to GP$\alpha$IIb$\beta$3 of the VWF released by ECs. Once immobilized on the membrane of adherent platelets, VWF promotes the recruitment of additional platelets supporting platelet-platelet aggregation with formation of platelet thrombi. Indeed, shear stress should be considered the most important regulator of the VWF binding to platelet receptors because high shear can unfold the globular VWF with formation of VWF fibers. Arterial thrombosis is the acute complication that develops on the chronic lesions of atherosclerosis. This may cause heart attack and stroke in a large number of individuals in developed countries. Plasma levels of HDL are negatively correlated with the incidence of atherosclerosis and the mechanisms of the antiatherogenic effects of HDL are mainly related to its involvement in the pathways of reverse cholesterol transport (RCT). ApoA-I, the major protein component of HDL, plays pivotal roles throughout the RCT process as follows: (1) formation and stabilization of HDL particle structure; (2) activation of lecithin cholesterol acyl transferase; (3) binding to the hepatic scavenger receptor (SRB1). ApoA-I exists in lipid-free, lipid-poor, and lipid-bound states and therefore has an adaptable structure. However, a lipid-free ApoA-I in full-length is crucial to understand HDL formation and atherosclerosis development.

In the last 2 decades, several authors have demonstrated in animal models the major roles of VWF in the localization of atherosclerotic and in the recruitment of platelets to atherosclerotic sites in response to hypercholesterolemia.

With this background information, Chung and colleagues demonstrated in vitro and in vivo using animal models that VWF self-association under shear stress can be modulated by HDL/ApoA-I, with significant reduction in length and thickness of VWF fibers. In their in vitro techniques, when they applied fluid shear stress in a flow chamber where cultured human ECs were activated, the hyperadhesive VWF strings were able to bind large numbers of platelets. If HDL was present during EC stimulation, fewer and shorter platelet-decorated VWF strings were formed. According to Chung and colleagues, this HDL effect on the reduced platelet deposition might be attributed to the binding of HDL particles to the scavenger receptor SRB1, activating EC nitric oxide synthase and inhibiting Weibel-Palade body secretion. The presence of HDL during both stimulation and platelet perfusion further reduced the number and length of platelet decorated strings. Their results obtained in vitro showed that HDL dampened VWF adhesive impact both by reducing its secretion and by interfering with its ability to form hyperactive strands. In another set of experiments, using endothelialized in vitro microvessels, they could demonstrate that ApoA-I prevents the incorporation of fluid-phase VWF multimers into preformed VWF fibers under flow but does not compete with platelets in the binding to these fibers.
To determine whether HDL could modulate VWF function in vivo, Chung and colleagues used a model of TMA induced by injections of high concentrations of human VWF into ADAMTS13-deficient mice. The protective effects of HDL on thrombocytopenia were accompanied by higher circulating VWF compared with the mice that received only VWF. These results may suggest that HDL stabilizes VWF in circulation, prevents the VWF self-association and binding to the vessel wall, and therefore reduces VWF-induced thrombocytopenia.

Based on these results obtained in both in vitro and in vivo models, the authors conclude that HDL/ApoA-I may have potent antithrombotic effects, clearly unrelated to reverse cholesterol transport (see figure). When HDL concentrations drop, the equilibrium shifts in favor of VWF self-association, especially in the setting of the rapid VWF secretion from the Weibel-Palade bodies. This unexpected and novel antithrombotic property of HDL/ApoA-I might have several important implications in both cardiovascular diseases and TMA as well as in sepsis, malaria, and sickle cell disease. The role of this novel antithrombotic effect of the HDL/ApoA-I in venous thrombosis needs further investigation.

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