

Signaling-mediated cooperativity between platelet GPIb-IX and PAR1. Depicted is a model for independent thrombin binding to 2 different platelet receptors that elicit mutually dependent signals via the 14-3-3-Rac1-LIMK1 pathway. See Figure 6i in the article by Estevez et al that begins on page 626.

(see figure). Rather, there is signaling cooperativity between GPIb-IX and PARs in the presence of low levels of thrombin. PAR-dependent responses require GPIb-IX, and GPIb-IX signaling requires cooperativity with PARs. The authors demonstrate the mutually dependent cooperativity signals via the 14-3-3-Rac1-LIMK1 pathway, which is also associated with von Willebrand factor signaling via GPIb-IX.⁷

The results presented with reconstituted platelet receptors on the surface of heterologous cell lines are compelling, but the important biologic question is whether low levels of thrombin are physiologically relevant during thrombus formation. To address this question, the authors conclude with a determination of in vivo thrombin levels at the site of a growing thrombus. Importantly, these data do document low levels of thrombin surrounding a growing thrombus consistent with the thrombin levels where the GPIb-IX/PAR cooperativity was observed. Thus, the in vivo result validates the importance of the data obtained using the heterologous cell model.

Although the work is performed and presented in a convincing manner, are there still unrecognized complexities that could challenge the proposed model? The experimental model of reconstituted platelet receptors in heterologous cells is certainly far removed from the anucleate platelet. Indeed,

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.⁸ 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 disrupts the 14-3-3-Rac1-LIMK1 signaling pathway in human platelets.⁹ However, there still could be subtleties 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 Estevez 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.¹⁰ 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.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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DOI 10.1182/blood-2015-12-683466

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● ● ● THROMBOSIS AND HEMOSTASIS

Comment on Chung et al, page 637

HDL/ApoA-I: role in VWF-dependent thrombosis

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In this issue of *Blood*, Chung and colleagues demonstrate in vitro and in an animal model that von Willebrand factor (VWF) self-association under shear stress can be modulated by the high-density lipoprotein and apolipoprotein A-I (HDL/ApoA-I)

complex, with significant reduction in the length and thickness of VWF fibers. These antiadhesive and antithrombotic properties of HDL/ApoA-I may connect the pathology of microvasculature with that of large vessels (atherosclerosis with arterial thrombosis) and might suggest novel approaches to these thrombotic disorders.¹

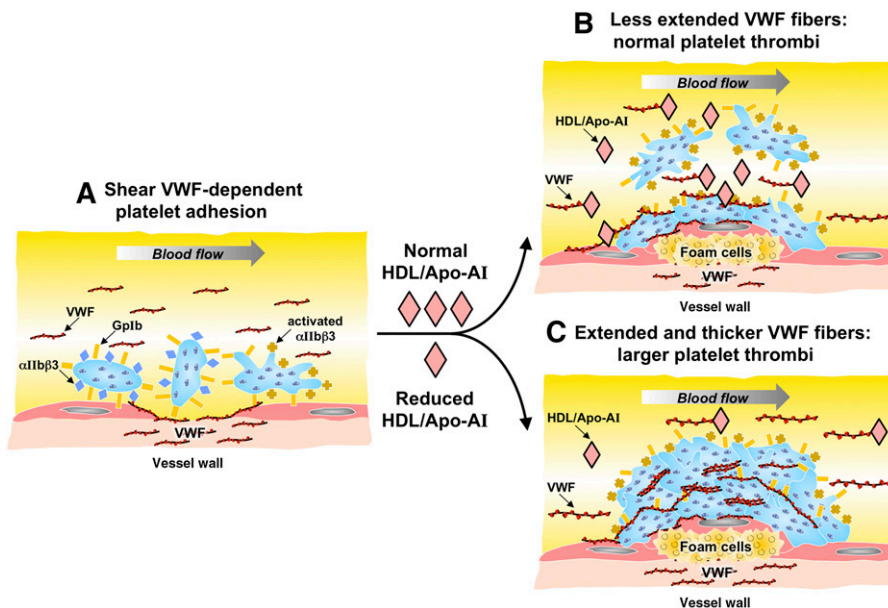
VWF is a multimeric glycoprotein that plays a major role in hemostasis and thrombosis by promoting adhesion to platelet receptors and platelet-platelet interactions under high shear stress conditions. VWF is synthesized by vascular endothelial cells (ECs) and megakaryocytes in ultra-large (UL) molecular weight multimers that can be stored in Weibel-Palade bodies and platelet granules.^{2,3} 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.^{2,3}

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 α IIb β 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.^{4,5} 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.⁶

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.⁴ 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.⁷ In the last 2 decades, several authors have demonstrated in animal models the major roles of VWF in the localization of atherosclerosis⁸ and in the recruitment of platelets to atherosclerotic sites in response to hypercholesterolemia.⁹

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 SRB1,⁷ 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.¹



During shear VWF-dependent platelet adhesion (A), the VWF A1 domain binds first to GPIIb; then, after initial tethering, the platelet becomes activated and promotes binding between VWF released from ECs and additional platelets, supporting thrombus formation. In the case of normal HDL/ApoA-I levels, VWF fibers are less extended with the normal size of platelet thrombi (B). In the case of reduced HDL/ApoA-I levels, VWF fibers are longer and thicker and generate larger platelet thrombi (C). These antithrombotic effects of HDL/ApoA-I are reported for the first time. Illustration by Luigi Flaminio Ghilardini, University of Milan.

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.¹

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.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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DOI 10.1182/blood-2015-12-682484

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