Blood Spotlight

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Platelets and von Willebrand factor in atherogenesis

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The role of platelet adhesion, activation, and aggregation in acute atherothrombotic events such as myocardial infarction and stroke is well established. There is increasing evidence that platelet-endothelial interactions also contribute to early atherosclerotic plaque initiation and growth. Through these interactions, platelet-derived factors can contribute to the proinflammatory and mitogenic status of resident mural cells. Among the many putative mechanisms for platelet-endothelial interactions, increased endothelial-associated von Willebrand factor, particularly in a multimerized form, which interacts with platelet glycoproteins and integrins, is a major factor and represents a therapeutic target in early atherogenesis. (*Blood*. 2017; 129(11):1415-1419)

Atherosclerotic disease develops over decades and involves many cell types throughout its course from plaque initiation to culmination in adverse atherothrombotic events such as acute myocardial infarction and stroke. Both the innate and adaptive inflammatory responses have well-recognized roles in atherosclerotic plaque growth and vulnerability and are mechanistically linked to the endothelial dysfunction, oxidative stress, cell migration, apoptosis, angiogenesis, and protease activity that determine the course of disease. The traditional view of platelets has been that they are primarily involved in precipitating acute thrombotic events in advanced disease. This construct has been challenged by preclinical research suggesting that cross-talk between hemostatic and inflammatory host defense mechanisms occurs at a much earlier stage of atherosclerotic disease development. A hallmark of early-stage atherogenesis is the compromise of normal endothelial function, which includes the actions of the endothelium to regulate platelet adhesion. Recent evidence indicates that platelets, which are armed with a secretome rich in proinflammatory mediators, are important contributors to early atherogenesis. In this focused review, we discuss the early atherogenic role of platelets as well as von Willebrand factor (VWF), which represents one of the putative mediators of platelet-endothelial interaction.

Platelet activation and adhesion in atherogenesis

The healthy endothelium in large vessels has multiple mechanisms that inhibit the adhesion or activation of platelets, either directly or by actively degrading platelet agonists. These actions are mediated by the release and/or cell-surface expression of nitric oxide, prostanoids (PGI2, PGE2), ectonucleotidases (CD39, CD73), adenosine, and thrombomodulin.[1-8] One of the earliest events in atherosclerosis is the loss of normal endothelial function, including disruption of antiplatelet mechanisms.[1-6] Later in this review, we discuss how abnormalities in VWF regulation are likely a contributing factor to atherosclerosis through the subsequent recruitment of platelets. However, other mechanisms may be operative that involve increased activation state of platelets triggered by classical risk factors of smoking, elevated low-density lipoprotein cholesterol, reduced high-density lipoprotein cholesterol, and insulin resistance.[9-11] Activation predisposes to platelet-leukocyte complexes mediated by P-selectin ligation of leukocyte PSGL-1, and CD18 integrin (ie, Mac-1) interaction with glycoprotein-Ibα (GPIbα) or with GPIIb/IIIa via fibrinogen.[7,12-14] There is evidence that transendothelial migration of platelet monocyte complexes may result in dissociation and surface deposition of platelets.[15] One of the most intriguing recent developments is the notion that local action of platelet microvesicles (commonly 100-200 nm in size) promotes vascular inflammation and lipid accumulation and is associated with development of subclinical lipid-rich atherosclerotic plaque.[16,17]

Preclinical studies have confirmed vascular adhesion of platelets in early atherogenesis. In hyperlipidemic rabbits and in apolipoprotein-E–deficient mice receiving a high-fat diet, direct endothelial adhesion of ex vivo–labeled platelets at lesion-prone sites has been observed early in atherosclerosis, the extent of which increased with plaque progression.[18-20] In vivo molecular imaging of a murine atherosclerosis has confirmed endothelial attachment of platelets even before the advent of a fatty streak.[21,22]

Proinflammatory platelets in atherosclerosis

There are many mechanisms by which presence of platelets can promote plaque initiation and progression.[4,14,23-26] One key process is the local release of platelet-derived proinflammatory factors, which include C-C motif chemokines (eg, RANTES, macrophage inflammatory protein-1β, CCL2), C-X-C motif chemokines (platelet factor-4, CXCL4, CXCL7, CXCL12), interleukins (IL-1β, IL-8), and CD40 ligand (CD40L). These factors promote a spectrum of proinflammatory effects, including monocyte activation, recruitment, and adhesion; upregulation of endothelial adhesion molecules; promotion of neutrophil extracellular traps; and uptake of oxidized low-density lipoprotein.[20,23-26] Some of these substances have been shown to be directly transferred from platelets to the endothelial or

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monocyte surface,[20,27] a process that may be receptor mediated.[28] Chemokine actions such as CD40-CD40L ligation stimulate the production and release of metalloproteinases, tissue factor, and reactive oxygen species (ROS),[29-33] all of which have a role in the predisposition to acute atherothrombotic events. Some of the strongest in vivo evidence of the proinflammatory atherogenic effects of platelets has been that functional inhibition of platelet activation or adhesion in animal models reduces plaque burden and plaque inflammation.[18,20,21] It has also been suggested that platelets play a protagonistic role in adverse proliferation of the vasa vasorum and plaque neovascularization, either directly through platelet-derived mitogenic growth factors or indirectly through the inflammatory response.[34-36] Platelets may also play a key role in neutrophil extracellular traps, which promote atherosclerosis progression.[37,38]

There are many processes that lead to self-amplification of further platelet recruitment in addition to the wide array of inflammatory chemokine receptors present on platelets.[25,39] Activation-related platelet release of VWF, ADP, and thromboxane A2 can lead to self-stimulation.[29,39,40] Oxidative stress is another important mechanism for self-amplification. ROS contribute to the loss of normal antiplatelet functions and VWF release[41]; however, platelets themselves generate ROS through NOX-1 and NOX-2.[42,43] Protein disulfide isomerase is another example, because this ubiquitous isomerase is secreted by platelets on activation, yet it also appears to augment the activity of NOX, thrombin, and platelet integrins.[44,45] Local thrombin production further amplifies ROS production through protease active receptors and GPIbα.[46] The self-amplifying nature of platelet adhesion makes this process an attractive therapeutic target. Recent studies of platelets from patients with hypercholesterolemia have demonstrated that statins that are routinely used for primary prevention of atherosclerosis reduce platelet activation status, platelet oxidative stress, and platelet collagen recruitment.[47] Recently, platelet $P2Y_{12}$ receptor antagonists have been shown to produce plaque-stabilizing effects.[48]

Role of VWF in atherogenesis

The relative importance of the different processes by which platelets or platelet microparticles interact with the intact endothelial surface and how they contribute to atherosclerosis lesion development continues to be a topic of active investigation. Like the situation with acute vascular injury, platelet recruitment in atherogenesis must be sustained under pulsatile, high shear stress conditions. Accordingly, molecular processes that are geared toward initial platelet recruitment under high hydrodynamic stress, such as catch-bond kinetics, are likely candidate mediators.[49]

Interaction between the GPIbα component of the platelet GPIb/IX/V complex and the active A1 domain of VWF, which does not require platelet activation, and integrin-mediated interaction with VWF represent classical pathways of platelet recruitment in hemostasis.[50] The concept that these processes participate in the initial stages of atherosclerosis is based on the idea that once they are adherent, platelets can be activated locally to release proinflammatory mediators and to recruit monocytes and other platelets. VWF is a large multimeric glycoprotein that is synthesized primarily by endothelial cells, is stored in Weibel-palade bodies in the form of multimerized homodimers, and undergoes enhanced release under conditions of endothelial cell activation.[51-54] Multimers undergo activation with gain of function of the A1 GPIbα-binding domain upon collagen attachment in high shear.[50] Under normal circumstances, VWF is cleaved at the Tyr1605-Met1606 site of the A2 domain by ADAMTS13 (A disintegrin-like and metalloprotease with thrombospondin type-1 repeats-13),[55,56] although other proteases may also contribute.[57] Ultralarge multimers of VWF that are not proteolytically cleaved by ADAMTS13 and contain active A1 domains can remain associated with the endothelial surface and potentially contribute to platelet-endothelial interactions in atherogenesis despite a high shear environment.[55] Release and formation of long VWF multimeric strings can be triggered by activated platelets via CD40L signaling, thereby representing another form of self-amplification.[58] Recent observations have strengthened the link between inflammatory processes involved in atherogenesis and VWF-mediated platelet recruitment. Fractalkine (CX3CL-1) has been shown to act synergistically to enhance VWF-mediated platelet rolling via GPIbα and adhesion via integrins.[59] Interaction with the complement component C1q represents an inflammation-related alternative mechanism for endothelial localization of VWF.[60]

With regard to human data, clinical trials have demonstrated that circulating levels of VWF are elevated in patients with recent acute coronary syndrome and that elevated VWF concentration is associated with higher risk for recurrent ischemic events and death.[61] Conversely, in large trials in those with atherosclerotic risk factors but without acute coronary syndrome, circulating levels of VWF are only modestly associated with risk for future atherosclerotic events or cardiac mortality.[61-63] These data would seem to argue that VWF becomes functionally important only in late atherothrombotic complications. However, circulating levels of VWF do not necessarily reflect events that occur at the blood pool–endothelial interface or the relative proportion of VWF in ultralarge multimers. In fact, preclinical data suggest a specific role for endothelial-associated VWF. In hypercholesterolemic rabbit models of early atherosclerosis, autoradiographic methods have demonstrated enhanced luminal VWF expression at lesion-prone regions, which spatially correlated with the site of platelet adhesion.[19] In these studies, functional inhibition of the VWF A1 domain or functional inhibition of platelet GPIbα markedly reduced platelet adhesion. Similarly, in apoliprotein-E–deficient atherosclerotic mice, functional inhibition of GPIbα reduced both transient and firm platelet adhesion.[18] In vivo ultrasound molecular imaging studies with pure intravascular probes designed to detect events at the endothelial surface have definitively demonstrated increased VWF signal on the intact endothelial surface in early-, mid-, and late-stage atherosclerotic disease in a murine model of progressive atherosclerosis.[22,64] This finding was associated with platelet-endothelial adhesion on molecular imaging and evidence for long VWF multimeric strings or nets on ex vivo microscopy.

The biologic basis for the apparent abundance of ultralarge VWF on the endothelial surface in early- to mid-stage atherosclerosis is probably multifactorial. Reduced ADAMTS13 availability or functionality leading to ineffective VWF cleavage has been a topic of investigation because these abnormalities are known to occur in inflammatory or prothrombotic states from either decreased ADAMTS13 synthesis[65,66] or increased proteolytic degradation.[67,68] On in vivo molecular imaging, high levels of platelet and VWF signal on the plaque surface in mice are eliminated by the exogenous administration of recombinant ADAMTS13.[22] There is also evidence that oxidative stress, which is an early contributor to atherosclerotic disease initiation, modifies the met1606 residue of VWF, making it less susceptible to cleavage.[69] Oxidative stress also modifies several regions of the functional site of ADAMTS13, rendering it less active.[70] These data implicating oxidative stress are congruent with findings that platelet-endothelial interactions in atherosclerotic mice are reduced by apocynin, which acts in part through inhibiting NOX.[21] Recent studies have also

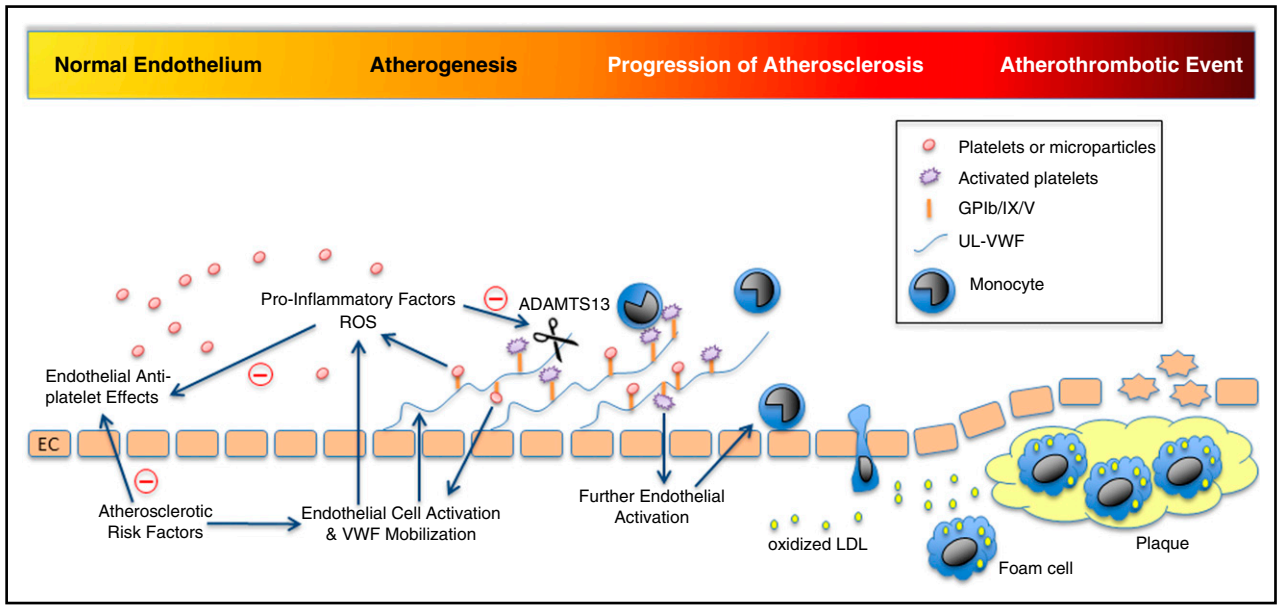

Figure 1. Schematic illustration of potential mechanisms and proatherogenic effects of platelet-endothelial interactions. EC, endothelial cell; LDL, low-density lipoprotein; UL, ultralarge.

demonstrated that high-density lipoprotein cholesterol prevents the self-association and multimeric assembly of VWF as well as platelet adhesion, thereby creating a mechanistic link between adverse atherosclerotic lipid profiles and platelet recruitment.[71]

Based on these considerations, it is likely that one of the earliest sequences of events in atherogenesis involves endothelial activation, release of VWF, which is not enzymatically cleaved and undergoes shear-dependent self-association and activation, and subsequent recruitment of platelets or platelet microparticles, which have important downstream proatherogenic actions (Figure 1). Because platelet-VWF interactions are sustained at high shear stress, this paradigm serves as a potential mechanism by which atherosclerosis is initiated in large arteries, and evidence exists that ligated platelets can directly support monocyte adhesion at high shear stresses.[12]

There is growing evidence that the abnormalities in platelet-endothelial interactions described here promote atherosclerotic lesion growth and susceptibility to atherothrombotic events. In murine models, atherosclerotic plaque size and inflammatory status are reduced by functional inhibition of GPIbα, genetic deletion of *GPIbα*, or genetic alteration of its functionality,[18,72,73] although not all studies have been consistent, with negative results also reported in mice lacking surface expression of GPIb-V-IX complex.[74] Similarly, the genetic deletion of VWF has also been shown to reduce plaque size, plaque spatial extent, and endothelial inflammation.[75] With regard to the specific role of ADAMTS13, genetic deletion of *ADAMTS13* in apoliprotein-E–deficient mice results in accelerated atherosclerosis, larger plaque size, and greater inflammatory cell content.[76,77] Perhaps the most compelling evidence of the importance of this pathway is that *ADAMTS13* deletion has a neutral effect on atherosclerotic lesion size and inflammatory cell content when combined with genetic deletion of VWF.[78] Together, these data provide a direct link between regulation of VWF, platelet recruitment, and early atherosclerotic lesion development.

Recent clinical studies in humans support the preclinical data. A case-control cross-sectional study demonstrated that the plasma levels of ADAMTS13 were lower in patients with significant atherosclerotic cardiovascular disease than in matched control participants.[79] More recently, it was reported that in those without a history of CAD who were followed for approximately a decade, lower levels of plasma ADAMTS13 activity were associated with higher rates of incident coronary events and cardiovascular mortality.[62,63] Activity of ADAMTS13 was more predictive than VWF antigen levels, thereby reinforcing the idea that examination of plasma VWF concentrations may not reflect regulatory processes occurring at the blood pool–endothelial interface.

Potential for new therapies

Drugs that are designed to reduce platelet activation and platelet aggregation are part of the well-established armament of therapies aimed at reducing atherosclerotic events. Although the primary purpose of these therapies is to reduce acute symptomatic atherothrombotic events, implicit in their use is that they may also reduce progression of plaque size or vulnerability by reducing the impact of subclinical thrombotic events.[80] The mounting evidence that platelet-endothelial interactions mediated by VWF contribute to early atherogenesis may lead to the development and clinical testing of new therapies. Because these same biologic events are common to the pathophysiology thrombotic thrombocytopenic purpura, it is quite possible that drugs designed to treat thrombotic thrombocytopenic purpura could also be applied to retard atherosclerosis. Enzyme supplementation with recombinant ADAMTS13 is unlikely to be useful, given the indolent nature of atherosclerosis. Better alternatives may be any one of the antibody, nanobody, aptamer, or small-molecule inhibitors of GPIbα or the VWF A1 domain.[81,82] Although early testing of these therapies is focused primarily on short-term treatment in acute coronary syndromes or stroke, inhibition of GPIbα has been shown to reduce ex vivo platelet adhesion to human plaque.[83] The main challenge in clinical testing of these approaches is the time and resource commitment to a long-term study investigating impact on atherogenesis and perhaps the

bleeding risk, which may be increased with therapies such as anti-VWF nanobodies (Caplacizumab).[84]

Conclusion

Ischemic complications of atherosclerotic disease, including acute atherothrombotic events, commonly come to clinical fruition after decades of indolent progression of disease. Current concepts of atherosclerotic plaque initiation and growth are based on the idea that maladaptation of common host defense mechanisms, in particular inflammatory vascular responses, occur in response to atherosclerotic risk factors. Platelets are increasingly recognized as multifaceted protagonists in the inflammatory response, and there are many potential mechanisms for their interaction with the endothelial surface. There is increasing evidence that VWF-mediated platelet recruitment plays an important role and may result from inadequate regulation of VWF at the endothelial surface. The importance of this specific pathway is the subject of ongoing investigation based on its potential as a therapeutic target.

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