ELSEVIER

Seminars in
HEMATOLOGY

New Perspectives on von Willebrand Factor Functions in Hemostasis and Thrombosis

Grazia Loredana Mendolicchio and Zaverio M. Ruggeri

The adhesive protein, von Willebrand factor (VWF), mediates the initiation and progression of thrombus formation at sites of vascular injury by means of specific interactions with extracellular matrix components and platelet receptors. The essential biologic properties of VWF have been elucidated, with progress particularly in the areas of genetic regulation, biosynthesis, and specific bimolecular interactions. The three-dimensional structure of selected domains has been solved, but our understanding of detailed structure-function relationships is still fragmented, partly because of the complexity and size of the VWF molecule. The biomechanical properties of the interaction between the VWF A1 domain and the platelet receptor glycoprotein (GP) Ibα also are better known, but we can still only hypothesize how this adhesive bond can oppose the fluid dynamic effects of rapidly flowing blood to initiate thrombus formation and contribute to platelet activation. Elucidating the details of VWF and GPIbα function will lead to a more satisfactory definition of the role of platelets in atherothrombosis, since hemodynamic forces greatly influence responses to vascular injury in stenosed and partially occluded arteries. Progress in this direction is also aided by rapidly expanding novel information on the mechanisms that regulate VWF multimer size in the circulation, a topic of relevance to explain microvascular thrombosis and, perhaps, arterial thrombosis in general. Developments in these areas of research will refine our understanding of the role played by VWF in vascular biology and pathology.
Semin Hematol 42:5-14 © 2005 Elsevier Inc. All rights reserved.

The response of platelets to vascular injury involves multiple vessel wall structures as well as cellular and soluble blood components. Among these, von Willebrand factor (VWF) and one of its platelet receptors, the glycoprotein (GP)Ibα in the GPIb-IX-V membrane complex,[1] can mediate platelet adhesion to extracellular matrices and to one another, opposing high shear stress, a hemodynamic condition present in areas of rapid blood flow such as microarterioles or stenosed arteries. This distinctive property makes VWF and GPIbα uniquely important for normal hemostasis and, presumably, central players in arterial thrombosis.[2] VWF also associates with the procoagulant factor VIII (FVIII), forming a complex that regulates FVIII secretion inside cells[3] and prevents its rapid clearance in the circulation.[4] The congenital deficiency of VWF function results in a bleeding tendency known as von Willebrand disease,[5] which occasionally may present as an acquired condition.[6] Comprehensive reviews on VWF have been published[7,8]; here, after a description of general concepts, attention will be focused on the structural and functional properties that characterize this unique prothrombotic molecule.

Biosynthesis and Structure of von Willebrand Factor

Mature VWF is a multimeric protein; molecules are composed of a variable number of identical subunits, each comprising 2,050 amino acid residues and up to 22 carbohydrate side chains.[9] Subunits are disulfide-bonded into dimers of approximately 500 kd, which in turn are disulfide-linked into multimers of increasing size that may exceed 10,000 kd. The largest VWF molecules have been visualized by electron microscopy as elongated or coiled filaments with lengths up to 1,300 nm (about the diameter of a platelet) or cross sections of 200 to 300 nm, respectively.[10] The size and shape of VWF molecules may influence their function, as discussed below.

Roon Research Laboratory for Arteriosclerosis and Thrombosis, Division of Experimental Thrombosis and Hemostasis, Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA.
Address correspondence to Zaverio M. Ruggeri, MD, The Scripps Research Institute, MEM-175, 10550 N Torrey Pines Rd, La Jolla, CA 92037. E-mail: ruggeri@scripps.edu

0037-1963/05/$-see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1053/j.seminhematol.2004.09.006

Biosynthesis and Secretion of von Willebrand Factor

The large VWF gene (180 kb) is located on the short arm of chromosome 12 and contains 52 exons. Expression is restricted to endothelial cells and megakaryocytes. The primary mRNA translation product, pre-pro-VWF, is a 2,813-residue polypeptide consisting of a 22-residue signal peptide, a 741-residue propeptide, and the 2,050-residue mature subunit. The propeptide and mature subunit constitute pro-VWF, composed of four types of repeating domains in the order D1-D2-D′-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2 from amino (NH_2)- to carboxyl (COOH)-terminal end.

After removal of the signal peptide from pre-pro-VWF and completion of the initial glycosylation,[11] pro-VWF monomers associate into dimers through intermolecular disulfide bridges within their COOH-terminal regions (residues 1,908 to 2,050).[12] Further processing of pro-VWF dimers is then followed by the noncovalent association of propeptide regions that promotes the assembly of larger multimers linked through disulfide bridges in the NH_2-terminal D3 domain. This step depends on the function of vicinal cysteine residues with endogenous disulfide isomerase-like activity[13] and is followed by the targeting of VWF multimers to storage granules.[14] In endothelial cells, VWF is stored in Weibel-Palade bodies that form only after VWF is synthesized but regardless of multimer assembly[15]; in megakaryocytes and platelets, VWF is stored in α-granules,[16] whose presence does not depend on VWF synthesis. The noncovalent propeptide dimer is removed by specific proteolysis before secretion and independently released into circulating blood, where it was originally identified as von Willebrand antigen II (VWAgII).[17] Two pathways of secretion, a constitutive one coupled to synthesis and a regulated one involving storage and release by secretagogues,[11] direct the release of endothelial cell–derived VWF both towards the lumen and the extracellular matrix. This polarity may be influenced by the nature of the release-inducing stimulus. In megakaryocytes and platelets only the regulated pathway is effectively operative in vivo; thus, the all of the VWF circulating in plasma is essentially of endothelial cell origin.[18]

Structure of von Willebrand Factor Functional Domains

Discrete domains within the VWF subunit exhibit specific functions, and several have been expressed as active fragments independently of multimer assembly. The recognized functional domains are D′, with a binding site for factor VIII and one for heparin; A1, with the binding site for platelet GPIbα as well as additional sites for collagens, heparin, sulfated glycolipids, and heterologous molecules such as the snake protein, botrocetin; A3, with an important binding site for collagens; and C1, with the integrin recognition sequence, Arg-Gly-Asp. Domain A2 does not contain a direct functional site but is important as the target of the metalloproteinase, ADAMTS13,[19,20] which cleaves specifically the Tyr842-Met843 peptide bond.[21] (Numbering is based on the mature subunit sequence beginning with Ser1, which is Ser764 of pre-pro-VWF where residue 1 is the initiating Met; thus, residues in the mature VWF subunit correspond to those in pre-pro-VWF after adding 763.) This cleavage has effects on the size of circulating multimers[22] and consequently modulates proadhesive functions (see below). Particular attention has been devoted to the VWF domains that promote platelet adhesion to extracellular matrix components, namely, A1 and A3, for which atomic structures have been obtained.

Structure of the VWF A1 Domain

The A1 domain, which contains the only known binding site for GPIbα, has a typical α/β fold with six hydrophobic parallel β strands forming a central β-sheet flanked by six α-helices, three on each side.[23] If one considers the A1 domain as a cube oriented such that the bottom face contains the intrachain disulfide bond formed by Cys509 and Cys695, then positively and negatively charged residues are clustered on the opposite right and left faces, respectively. Residues on either side of the disulfide bond are packed against the bottom face of the domain by hydrophobic or salt-bridge interactions. The structural bases of VWF A1–GPIbα binding have been elucidated by the crystallization of two complexes, the first containing gain-of-function mutations in both A1 domain (Arg543Gln) and GPIbα amino terminal domain (Met239Val),[24] and the more recent one obtained with wild-type molecules.[25] The difference in binding affinity between the two complexes is approximately 2.5-fold. Both structures indicate that the GPIbα binding surface is discontinuous and involves two areas: the larger one, located on the upper-front face of the A1 domain, includes helix α3, loop α3-β4, and strand β3; the smaller one, located on the bottom face, includes loops α1-β2, β3-α2, and α3-β4. The two crystal structures differ substantially in the position of the α1-β2 loop of the VWF A1 domain, a difference that is not observed in the uncomplexed native and mutant molecules but only upon their binding to GPIbα.[25] In the bound mutant A1 domain, the α1-β2 loop has a more extended conformation and protrudes towards the interior of the GPIbα binding pocket, which may lead to more electrostatic interactions between receptor and ligand and therefore a tighter bond. In contrast, in the bound wild-type A1 domain, the loop has a more open conformation and is not as close to GPIbα residues as the corresponding loop in the mutant A1 domain, such that several water molecules are incorporated at the contact interface.[25] Different crystal structures of uncomplexed A1 domain have revealed several conformations that appear to be intermediate between the open one of the bound wild-type and the extended/closed one of the bound Arg543Gln mutant. This suggests that conformational adaptations of the α1-β2 loop may be responsible for affinity modulation of VWF A1–GPIbα binding.

Functional Modulation of the VWF A1 Domain

Native VWF in solution has no measurable affinity for GPIbα, a condition that is thought to prevent binding to platelets in circulating blood. Mechanisms exist that promote the interaction at sites of vascular injury in order to initiate thrombus formation. As described above, one such mechanism may involve conformational changes in the α1-β2 loop located at the bottom face of the A1 domain. In this region of the molecule, residues on the NH_2- and COOH-terminal sides of the Cys509-Cys695 disulfide bond may mask an area that contributes to the interaction with the GPIbα. In native VWF, therefore, neighboring domains may impede A1 binding to platelets until the functional site is exposed through conformational rearrangements. In this regard, most gain-of-function mutations identified in type 2B von Willebrand disease[26] are clustered at the bottom face of the A1 domain. Mutations in this region may disrupt the salt-bridge or hydrophobic interactions that contribute to maintaining a nonfunctional closed conformation, thus enhancing GPIbα binding. In the case of the Ile546Val type 2B mutation, the crystal structure reveals structural changes propagated at a distance of 27 Å from the α1-β2 loop, where the amino acid substitution occurs, and reflected mainly in reorientation of residues Asp560 and Gly561 that are part of the upper-front GPIbα-binding surface.[27] The extent to which such changes contribute to the gain of function is still unclear, but the effects of these mutations are compatible with the concept that GPIbα binding involves an extended surface of VWF A1. Moreover, at least one type 2B mutation, Arg543Gln, resulted in structural alterations that became more apparent upon binding to GPIbα,[25] highlighting the adaptive and dynamic nature of the interaction. In pathophysiologic situations, the two relevant conditions that may influence the VWF A1–GPIbα binding affinity, often acting in concert, are immobilization on a substrate and elevated fluid shear stress.[28] Both may contribute to the exposure of functional sites, as suggested by the occurrence of molecular shape changes induced by fluid dynamic forces in surface bound VWF.[29] Perhaps less relevant for a direct understanding of the physiologic modulation of A1 domain function is the information provided by the use of heterologous modulators such as ristocetin[30] and botrocetin,[31] a bacterial glycopeptide and snake protein, respectively. Both bind to the A1 domain: botrocetin to positively charged residues located on the right-hand face of the molecule,[32] and ristocetin likely to a sequence including three consecutive proline residues (702 to 704) that flank Cys695 on the COOH-terminal side.[33] There is no evidence to date that these modulators act in a manner that reflects mechanisms relevant for biologic functions; yet, it must be noted that ristocetin-induced VWF–platelet interactions rather faithfully reflect the dysfunction caused by VWF mutations in normal hemostasis.

Structure of the VWF A3 Domain in Relation to Collagen Binding

There is general agreement that the VWF A3 domain contains a binding site for fibril-forming collagens (types I and III), whereas there is more controversial evidence on the contribution provided to collagen binding by the A1 domain.[7] The latter may be particularly important for binding to collagen type VI34, which is an important component of the subendothelial matrix. Fluid dynamic conditions and mechanical forces may modulate VWF-collagen interactions, and domains A1 and A3 may variably contribute to the immobilization of VWF onto extracellular matrices. This concept may explain the discrepant observations that an anti-VWF A3 domain antibody blocking binding to collagen had antithrombotic effects,[35] unlike mutations such as Ser968Thr that markedly inhibited the same interaction in vitro but had limited effects in vivo.[36] Thus, the immobilization of VWF onto extracellular surfaces mediated by the A3 domain may be variably relevant depending on the nature of the vascular lesion.

Determination of the crystal structure of the A3 domain has revealed a folding similar to that of the A1 domain.[37,38] The putative location of collagen-binding residues was deduced from the structure of the A3 domain in complex with a function-blocking monoclonal Fab capable of inhibiting collagen binding.[39] The functional site may be located at the bottom face of the molecule, although mutagenesis studies have also indicated a role for residues on the front face.[40] Experiments with transferred cross-saturation, a technique based on nuclear magnetic resonance that allows the visualization of contact residues in large complexes, identified the front face of the A3 domain as the site for collagen binding.[41] This function of VWF A3 appears to be constitutively expressed and independent of specific conformational changes, although it may involve an induced-fit mechanism.[39]

Functions of von Willebrand Factor in Platelet Adhesion and Aggregation

Platelet Thrombus Formation in Flowing Blood

The complex interactions that involve cells, the vessel wall and molecules in solution during a normal hemostatic response are influenced by the flow of blood. The velocity of blood near the wall is lower than at the center of the vessel, and this difference creates a shearing effect between adjacent layers of fluid that is greatest at the luminal surface, decreasing progressively towards the center of the vessel (Fig 1). Shear rate, a difference in flow velocity as a function of distance from the wall, is expressed in cm/s per cm or the equivalent inverse seconds (s^{-1}). Fluid shear stress is a force per unit area and is expressed in Pascal (Pa), equivalent to 1 N/m^2 or in dynes/cm^2 (1 Pa = 10 dynes/cm^2). The shear rate is directly proportional to the shear stress and inversely proportional to the fluid viscosity. In blood, where viscosity is approximately 0.004 Pa · s (or 0.04 dynes/cm^2 · s), a shear stress of 1 dyne/cm^2 corresponds to a shear rate of 25 s^{-1}. The force (drag) that opposes stable platelet adhesion and aggregation increases with the shear rate. Consequently, shear-depen-

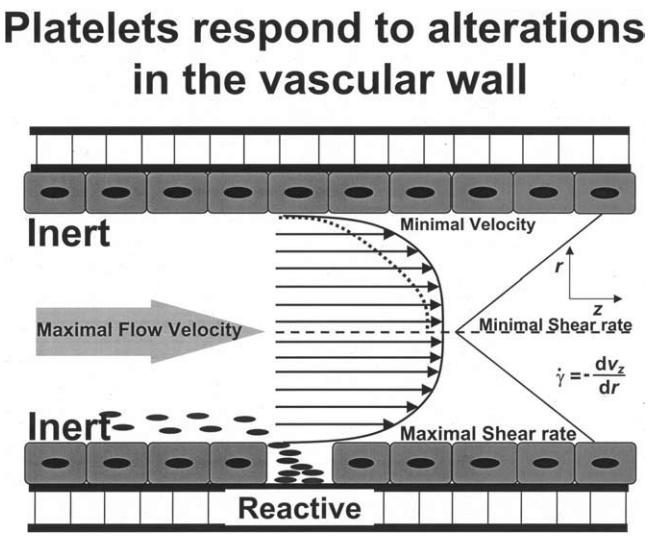

Figure 1 Normal endothelial cells are nonreactive for platelets, but exposed subendothelial structures induce rapid platelet adhesion. Hemodynamic forces influence these events in flowing blood. Blood flow in a cylindrical vessel can be visualized as a series of fluid layers (laminae) moving at different velocity. The laminae near the center of the vessel have greater velocity than those near the wall (depicted schematically by arrows of different length). The corresponding velocity profile (solid line) is more blunted than the parabolic profile expected with a homogeneous suspension (dotted line) owing to cell depletion in the boundary layer near the wall. The shear rate is the rate of change of velocity with respect to distance measured perpendicularly to the direction of flow. The negative sign indicates that the gradient is defined from the center (where velocity is maximal) to the wall (where velocity is minimal).

dent phenomena are more relevant in those districts of the vasculature where shear forces are greater (that is, in arteries more than in veins) and, particularly, in arterioles. The highest wall shear rate in the normal circulation occurs in small arterioles of 10 to 50 μm diameter where levels have been estimated to vary between 500 and 5,000 s^{-1}. Wall shear rates of 3,000 to 10,000 s^{-1} have been measured at the top of plaques causing 50% occlusion of coronary arteries,[42] and values in excess of 50,000 s^{-1} have been estimated to occur at the apex of a more severe stenosis.[43,44] Thus, shear stress is of greater pathogenic relevance in conditions predisposing to acute arterial occlusion than it is in the course of normal hemostasis.

Regulation of VWF Multimer Size in the Circulation and Its Relationship to Thrombogenesis

The largest VWF multimers display enhanced thrombogenic functions, possibly because multiple interactive sites for vessel wall components and platelets support more efficient adhesion. The most active multimers are normally present in storage granules within cells and are not seen in blood, where they can be detected as unusually large multimers only transiently after induction of secretion from endothelial storage sites.[45] The controlled release of the largest VWF multimers at the time of injury limits their presence at sites of tissue damage, and physiologic regulatory mechanisms cause their disappearance from the circulation, possibly to prevent excessive thrombus formation. The main mechanism regulating plasma VWF size involves specific proteolysis by ADAMTS13.[46,47] Mutations of ADAMTS13 have been identified in patients with chronic relapsing thrombocytopenic purpura,[46] a discovery that correlates the altered processing of VWF multimers in blood to the microvascular thrombotic complications typical of this disorder.[48] Deficiency of ADAMTS13 activity has also been correlated to enhanced shear-induced platelet aggregation,[49] which may explain the preferential onset of thrombosis in small arterioles where shear stress is elevated. ADAMTS13 can cleave newly secreted ultralarge VWF multimers on the surface of endothelial cells,[50] and a defect in this function may influence the local progression of prothrombotic events.[51] There is an obvious interest in determining whether a similar mechanism might contribute to precipitating acute thrombotic complications of coronary artery and cerebrovascular disease, and more studies will undoubtedly be devoted to address this question. In addition to proteolysis, the regulation of VWF multimer size may depend on a disulfide bond reductase activity ascribed to thrombospondin-1. In this process, disulfide bonds linking VWF subunits are rearranged such that the largest multimers are "depolymerized," reversing the assembly achieved during biosynthesis.[52]

Initial Platelet Attachment to Thrombogenic Surfaces

Distinct ligand-receptor interactions initiate thrombogenesis at sites of vascular injury depending on the velocity of flowing blood. Above a limiting shear rate (~1,000 s^{-1}) the process is absolutely dependent on VWF and its GPIbα receptor, even on substrates such as collagen and complex extracellular matrices that are intrinsically capable of initiating adhesion at lower shear rates[53] (Fig 2). The distinguishing feature of the VWF A1 domain–GPIbα interaction is the ability to support platelet tethering to thrombogenic surfaces even when the velocity of flowing blood relative to the vessel wall is elevated. However, the interaction is also characterized by a fast dissociation rate and cannot support irreversible adhesion,[54] so that platelets tethered in such a manner to the vessel wall move constantly in the direction of flow (Fig 2). The translocation velocity is typically less than 2% of the free flow velocity of noninteracting platelets at the same distance from the luminal surface. This slow motion allows the establishment of additional bonds through receptors that belong mostly, but not necessarily, to the integrin superfamily. Such receptors typically have a slower rate of bond formation as compared to GPIb but are capable of mediating stable interactions that lead to the definitive arrest of individual platelets and subsequent thrombus development. Notable in this regard is the role of the activated integrin αIIbβ3, which can bind to the Arg-Gly-Asp sequence in VWF itself[55,56] or to other adhesive substrates in a complex matrix, and of collagen and its receptors.[53,57,58] When VWF is bound to collagen,

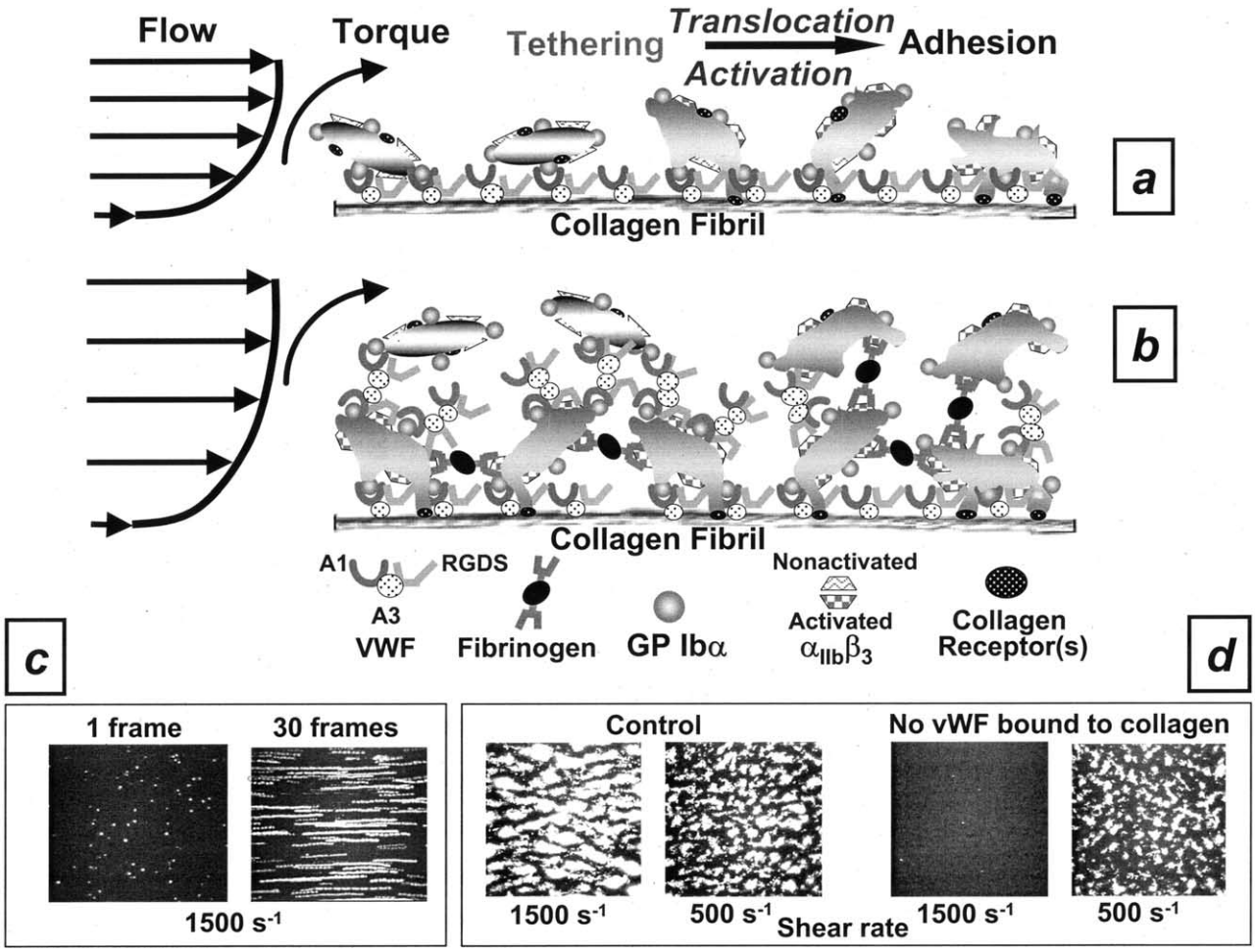

Figure 2 Schematic representation of the sequential process of platelet adhesion and thrombus formation onto collagen type I fibrils under a high shear rate. (*a*) VWF bound to collagen is required to establish the first contact between platelets and the surface when the shear rate exceeds a threshold value (~1,000 s^{-1} with human blood). The VWF A1–GPIbα bonds forms rapidly and have high resistance to tensile stress but an intrinsically limited half-life. In the absence of other bonds, platelets detach at the tailing edge where tension is greatest and move forward with a rotational movement (rolling) due to the torque imposed by the flowing fluid. New VWF A1–GPIbα bonds form as different regions of the membrane of rolling platelets come close to the surface, thus platelets remain in contact with the substrate for extended periods of time while translocating at low velocity. In normal conditions, however, the initial tethering to VWF is rapidly followed by binding to collagen through specific receptors (namely, GPVI and $\alpha_2\beta_1$), followed by firm adhesion, activation, and additional stable bonds mediated by $\alpha_{IIb}\beta_3$. (*b*) The first layer of activated platelets firmly attached to a reactive surface becomes the substrate for accumulation of more platelets and thrombus growth. Adhesive ligands, mainly fibrinogen and VWF, bind to activated $\alpha_{IIb}\beta_3$ on the membrane of the adherent platelets and become the substrate for the attachment of incoming platelets. The unique biomechanical properties of the VWF A1–GPIbα bond are required for the initial tethering of flowing platelets to adherent platelets when the shear rate is elevated. This explains why in rapidly flowing blood VWF and GPIbα play a key role both during initial platelet adhesion and subsequent aggregation. (*c*) Visualization of platelet rolling on immobilized VWF by time-lapse videomicroscopy. Blood containing as anticoagulant the thrombin inhibitor D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone dihydrochloride (PPACK; 40 μmol/L final concentration) as well as prostaglandin E_1 to inhibit platelet activation was perfused through a parallel plate flow chamber at 37°C with a wall shear rate of 1,500 s^{-1}. Platelets were rendered fluorescent by the incorporation of mepacrine. The image on the left is a single frame showing a snapshot of individual platelets interacting with the VWF-coated surface. The image on the right shows the superimposition of 30 consecutive frames captured a sampling rate of three frames per second. The occurrence of platelet translocation on VWF is rendered by the streaking effect produced by moving platelets. (*d*) VWF is required for thrombus formation on collagen when the shear rate is high but not when it is low. The two images on the left show the thrombi formed when control blood was perfused over collagen type I fibrils at two different shear rates. The two images on the right show the thrombi formed when the same blood was treated before perfusion with an anti-VWF A3 antibody that blocks binding to collagen. Thrombus formation was abolished when the shear rate was 1,500 s^{-1} but minimally affected when the shear rate was 500 s^{-1}.

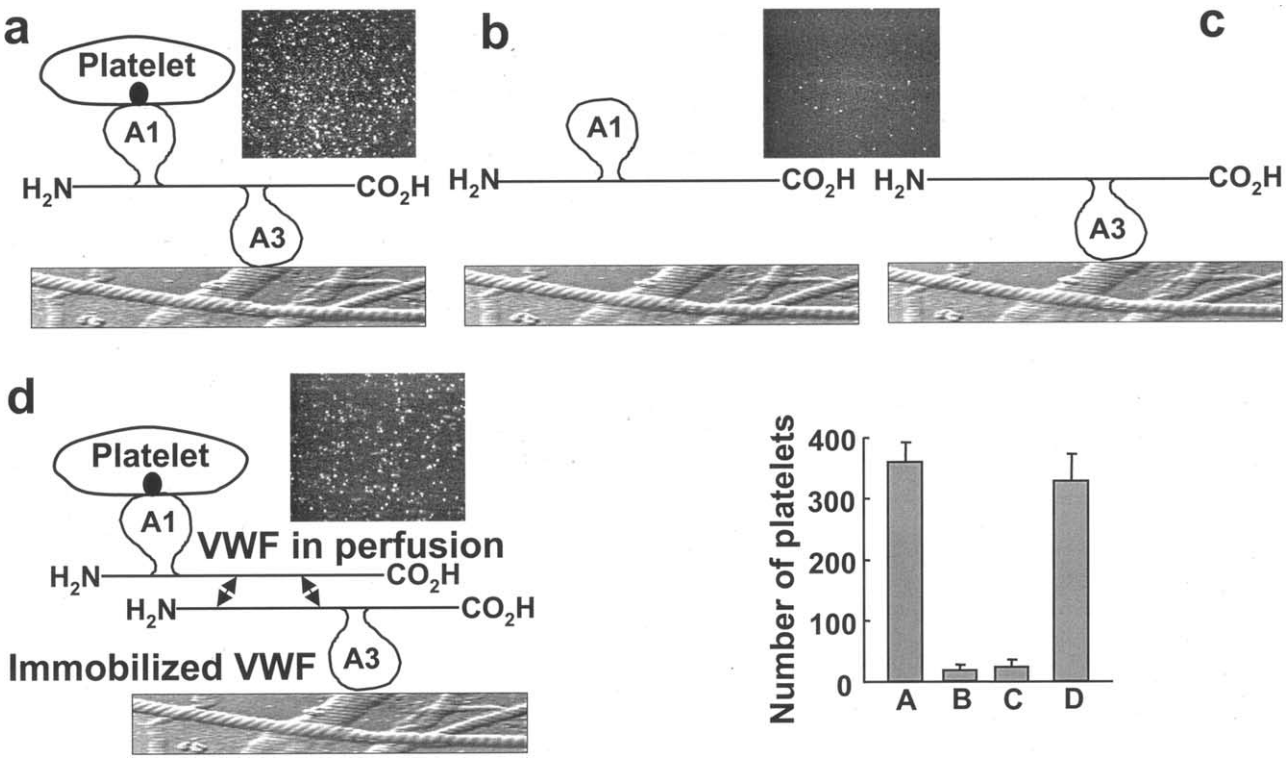

Figure 3 Role of soluble VWF A1 domain in mediating platelet interaction with surface-bound VWF. A washed blood cell suspensions devoid of plasma proteins and containing EDTA, to block integrin function in adhesion and aggregation, and prostaglandin E_1, to block platelet activation, was perfused over immobilized collagen type I fibrils at the wall shear rate of 1,500 s^{-1}. (a) Control experiment with normal multimeric VWF added to the cell suspension. The A3 domain mediates VWF binding to collagen, and the A1 domain interacts with platelet GPIbα; tethered platelets are seen rolling on the surface, which is represented by an electron micrograph of collagen fibrils. (b) Experiments performed after adding to the cell suspension recombinant VWF devoid of the A3 domain (ΔA3-VWF), which cannot bind to collagen; or (c) recombinant VWF devoid of the A1 domain (ΔA1-VWF), which binds to collagen but cannot interact with platelet GPIbα. In either case, no platelets are seen tethered to the surface. (d) The collagen fibrils were precoated with ΔA1-VWF multimers, which cannot initiate platelet tethering, and then exposed to the blood cell suspension containing ΔA3-VWF. Although the latter cannot bind directly to collagen (see b), it could compensate for the lack of A1 domain in the surface-bound VWF and restore platelet tethering. The association of VWF multimers with one another can explain this result; the two-sided arrows between multimers indicate that the association is reversible. The images are single frames from a real time recording representing an area of 65,536 μm^2. The bar graph shows the number of platelets tethered to the surface under the different experimental conditions described above (mean $\pm$ SEM of two separate experiments). Adapted from Savage et al[59] and reprinted with permission.

the transition from rolling to stable adhesion occurs much more rapidly than on immobilized VWF alone, indicating the effect of multiple activating signals.

An additional mechanism has been elucidated that highlights how soluble plasma VWF may contribute to platelet adhesion.[59] A process of self-association occurs at the interface between soluble and surface-bound VWF, such that the first layer of VWF multimers bound to matrix collagen supports the transient adsorption of additional multimers from plasma to which platelets can readily attach. This mechanism was demonstrated by binding to collagen a mutant VWF devoid of domain A1, thus unable to promote platelet adhesion, and showing that GPIbα-mediated tethering was restored by the presence of soluble VWF in plasma (Fig 3). The adsorption of soluble VWF to immobilized VWF is a reversible process since the number of platelets-surface contacts on immobilized ΔA1-VWF is rapidly reduced when the pefusing blood is replaced with a plasma-free blood suspension.[59] Plasma VWF, therefore, has a dual role in mediating platelet adhesion under flow: one involves the A3 domain through which tight binding to collagen is established, and the other is a reversible self-association with immobilized VWF that can modulate and reinforce the adhesive properties of the latter. These findings demonstrate that a direct linkage between VWF and a surface such as collagen is not an absolute requisite for the expression of GPIbα binding function.

The VWF–GPIbα Interaction in Arterial Disease

In diseased arteries, the tethering of flowing platelets to exposed VWF may precipitate the thrombotic occlusion of the vessel lumen with obstruction to blood flow and subsequent tissue damage.[2] Shear stress is particularly elevated around

atherosclerotic lesions because lumen restriction causes a local increase in blood flow velocity. High shear stress may specifically enhance platelet reactivity through the VWF–GPIb-IX-V pathway of platelet adhesion and activation.[60,61] Clinical and experimental evidence may link VWF to acute arterial thrombosis. For example, diabetes is an established risk factor for cardiac death, and increased levels of VWF are significantly and independently associated with diabetes in patients who have suffered a myocardial infarction.[62] Moreover, VWF-mediated platelet adhesion to the vessel wall may also contribute to the genesis and progression of atherosclerotic plaques, which represent the chronic vascular alteration onto which acute arterial thrombosis develops. In this regard, enhanced secretion of VWF in response to inflammatory stimuli can lead to the local recruitment of platelets, and the process may be favored by hypercholesterolemia.[63] This may be one of the reasons why deficiency of VWF affords some level of protection from atherosclerosis.[64]

VWF-Mediated Signaling Through GPIbα and Platelet Activation

The binding of the VWF A1 domain to GPIbα elicits signals—including elevation of cytosolic Ca^{2+} and activation of protein kinase C and tyrosine kinases—that may contribute to platelet activation. These signals can be detected under static conditions,[65,66] but they may be particularly relevant for the activation of platelets exposed to high shear stress.[67] Two distinct elevations of intracytoplasmic Ca^{2+} have been documented in association with platelet GPIbα-mediated adhesion to immobilized VWF (Fig 4). A first rise in $[Ca^{2+}]_i$, designated an α/β peak, occurs during the initial contacts that are still potentially reversible and always precedes stable platelet attachment. Type α/β peaks involve Ca^{2+} release from intracellular stores. A second Ca^{2+} signal, designated a γ peak, occurs in platelets that have established firm adhesion through the integrin αIIbβ3 and appears to be required for subsequent aggregation. Type γ peaks involve a transmembrane Ca^{2+} flux. Thus, the initial GPIbα interaction with the VWF A1 domain leads to first-level αIIbβ3 activation sufficient for stable platelet adhesion to immobilized VWF but not for binding soluble VWF or fibrinogen (required for aggregation). Progression to thrombus formation requires further αIIbβ3 activation that is contingent on signal amplification associated with type γ $[Ca^{2+}]_i$ peaks (Fig 4). Inhibition of phophatidylinositol 3 kinase (PI3-K) activity or removal of adenosine triphosphate (ADP) with apyrase blocks γ peaks and prevents platelet aggregation.[67,68] While apyrase has no effect on the frequency or amplitude of α/β peaks, the use of specific inhibitors highlights an important role of the $P2Y_1$ ADP receptor in reinforcing the initial signal induced by GPIbα binding to VWF A1. Thus, the latter interaction causes ADP release that acts rapidly through $P2Y_1$ in enhancing Ca^{2+} release from intracellular stores, such that enzymatic degradation of ADP by apyrase is not sufficiently rapid to block this activity.[69] In contrast, a subsequent effect of ADP required for platelet aggregation and mediated by a

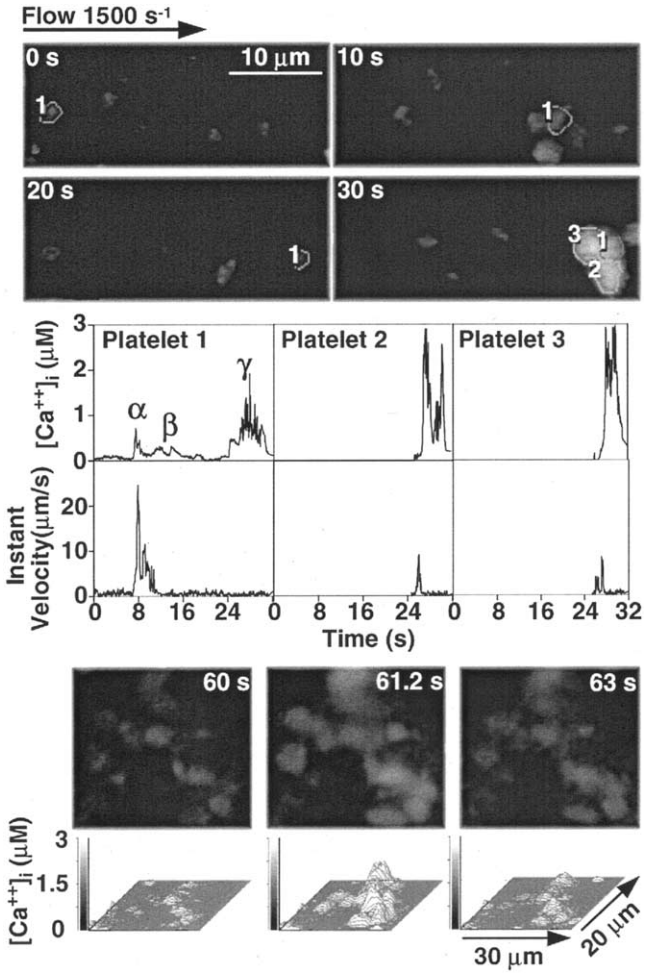

Figure 4 Real-time analysis of $[Ca^{2+}]_i$ during platelet translocation and aggregate formation on immobilized VWF. Platelets loaded with acetoxymethyl ester derivative of fluo-3 (2×10^7/mL) were suspended with washed erythrocytes in homologous plasma and perfused over immobilized VWF for 3 minutes at the shear rate of 1,500 s^{-1}. The sequence of images at the top shows an example of aggregate formation. At 0 seconds, platelet 1 appears in the optical field; at 10 seconds, it has moved in the direction of flow by approximately 20 μm; at 20 seconds, it has moved by an additional few μm; at 30 seconds, it is in the same position, and two new platelets (2 and 3) are attached in close proximity forming a small aggregate. The diagrams in the middle show $[Ca^{2+}]_i$ and instant velocity of platelets 1, 2, and 3. The translocation of platelet 1 occurs mostly during a few seconds of relatively rapid movement, coincident with the appearance of transient $[Ca^{2+}]_i$ peaks (α/β); a higher and longer lasting increase in $[Ca^{2+}]_i$ (γ) develops while the platelet is stationary. Cytosolic Ca^{2+} oscillations appear also when platelets 2 and 3 arrest on the surface, without a clear sequence from α/β to γ. The images at the bottom, captured between 60 and 63 seconds after the appearance of platelet 1 in the field, show the long-lasting synchronous increase of $[Ca^{2+}]_i$ in platelets forming a large aggregate. The three-dimensional diagrams below each image show the measurement of $[Ca^{2+}]_i$ in all the platelets in the field. Adapted From: Mazzucato et al. Sequential cytoplasmic calcium signals in a two-stage platelet activation process induced by the glycoprotein Ibα mechanoreceptor. *Blood* 2002;100:2793-2800. Copyright American Society of Hematology, used with permission.

different receptor, $P2Y_{12}$, can be blocked efficiently by apyrase.[69]

Signaling dependent on the VWF A1 domain is thought to involve the cross-linking (receptor clustering) of GPIbα subunits by multivalent VWF.[65] The observation that a subpopulation of platelet GPIb-IX-V is associated with FcRγ-chain, which contains an ITAM (immuno-receptor tyrosine-based activation motif) module, lends support to the cross-linking mechanism of signaling.[70–72] FcRγ-chain and GPIbα are proximal to within 10 nm of each other in the platelet membrane.[73] Nevertheless, VWF A1 domain–induced signaling through GPIbα can activate αIIbβ3 in a manner that is independent of other receptors.[66] In particular, mouse platelets expressing human GPIbα but lacking the FcRγ-chain adhere to a dimeric VWF A1 domain and undergo Ca^{2+} oscillations in a manner that is qualitatively similar, albeit slightly impaired, to that seen with mouse platelets expressing both receptors. Thus, the FcRγ-chain contributes to maximal Ca^{2+} elevations, but it is not strictly required for the initiation of Ca^{2+} responses.

Thrombus Propagation Under Flow

The response of platelets to vascular injury involves a series of coordinated reactions initiated by tethering to appropriate substrates linked to activation processes that promote the binding of soluble adhesive proteins and the recruitment of additional platelets. The time course of this sequence of events is influenced by the composition of the reactive surface exposed to flowing blood. In the setting of vascular lesions, subendothelial components such as collagen and soluble platelet agonists such as ADP, epinephrine, and α-thrombin may greatly enhance the efficiency of the hemostatic response by contributing synergistically to αIIbβ3 activation. It is apparent that soluble agonists may influence thrombus formation at sites of injury, but in the early stages of the process a relevant role is likely played by activation signals originating from the engagement of adhesion receptors. Indeed, irreversible platelet adhesion to matrices containing collagen type I is essentially instantaneous even at the highest levels of shear stress, and thrombus formation ensues within seconds.[53]

After platelets adhere to a vascular lesion and become activated, thrombus growth proceeds through local accumulation of additional platelets in a process of homotypic aggregation. During this phase, activated adherent platelets bind soluble adhesive proteins, mainly fibrinogen and VWF, which upon immobilization onto the cellular membrane become substrates for the adhesion of circulating platelets in successive layers.[53] VWF released from activated platelets may play a role at this stage by increasing the local ligand concentration and membrane binding. Activation of the newly recruited platelets continues the process until the thrombus mass grows sufficiently to arrest blood loss from an injured vessel or, in pathologic conditions, until regulatory mechanisms interrupt the cycle or the vascular lumen is occluded. Thus, notwithstanding the importance of initiating events, a thrombus is constituted essentially of platelets linked to one another but not directly to subendothelial components, and its development is strictly dependent on the formation of interplatelet bonds.

Fibrinogen bound to activated αIIbβ3 is generally considered the predominant adhesive bridge between aggregating platelets.[74] At elevated shear rates, typically in excess of 5,000 s^{-1}, VWF can also bind to platelets in a process that involves sequentially GPIbα and αIIbβ3 and is thought to mediate aggregation in alternative to fibrinogen.[28,75] Such concepts, however, have been suggested by the results of experiments with platelets in suspension not interacting with a reactive substrate. Studies on the bonds that link platelets to one another during thrombus development on collagen type I fibrils indicate a synergistic role for fibrinogen and VWF in supporting platelet aggregation. In the absence of fibrinogen, VWF-mediated thrombi grow rapidly at high shear rates but are unstable; in the presence of VWF and fibrinogen, thrombi grow more slowly, but are stable.[61] Unstable thrombi in the absence of fibrinogen have also been seen in the mouse circulation.[76] These results provide a plausible explanation for the altered hemostatic properties of platelets from patients with isolated congenital deficiency of either fibrinogen or VWF. Because neither protein by itself can sustain the full development of stable thrombi within the range of pathophysiologically relevant flow conditions, hemostasis cannot be normal unless both are present and functional. Studies in genetically modified mice have indicated that thrombus formation may persist even in the absence of both fibrinogen and VWF,[77] and evidence has been obtained that fibronectin, another ligand for activated αIIbβ3, may contribute to platelet aggregation within a thrombus.[78] In this regard, therefore, it appears that several αIIbβ3 ligands support thrombus stability, including CD40 ligand.[79]

The role of different adhesion receptors in mediating interplatelet contact has been analyzed with two-step perfusion experiments.[61] These studies have shown that thrombus height is limited by the inhibition of GPIbα even when blood is initially perfused at shear rates permissive of adhesion to collagen without participation of the GPIbα–VWF interaction. This is because the shear rate increases at the apex of a growing thrombus where the flow channel narrows, such that the threshold may be reached at which only a rapidly forming bond can capture flowing platelet. Thus, the synergy between fibrinogen and VWF in supporting platelet aggregation depends on the recognized ability of each of these molecules to establish bonds with distinct adhesive properties.

Conclusions

Progress in understanding the structure and function of VWF and the mechanisms that underlie its interactions with vascular surfaces and platelets can elucidate important aspects of normal hemostasis and pathologic arterial thrombosis. This knowledge may translate into better treatments for rare as well as common diseases, such as thrombotic thrombocytopenic purpura and myocardial infarction.

References

1. Andrews RK, Gardiner EE, Shen Y, Whisstock JC, Berndt MC: Glycoprotein Ib-IX-V. Int J Biochem Cell Biol 35:1170-1174, 2003
2. Ruggeri ZM: Platelets in atherothrombosis. Nat Med 8:1227-1234, 2002
3. Rosenberg JB, Foster PA, Kaufman RJ, Vokac EA, Moussalli M, Kroner PA, et al: Intracellular trafficking of factor VIII to von Willebrand factor storage granules. J Clin Invest 101:613-624, 1998
4. Nogami K, Shima M, Nishiya K, Hosokawa K, Saenko EL, Sakurai Y, et al: A novel mechanism of factor VIII protection by von Willebrand factor from activated protein C-catalyzed inactivation. Blood 99:3993-3998, 2002
5. Castaman G, Federici AB, Rodeghiero F, Mannucci PM: Von Willebrand's disease in the year 2003: Towards the complete identification of gene defects for correct diagnosis and treatment. Haematologica 88:94-108, 2003
6. Federici AB, Budde U, Rand JH: Acquired von Willebrand syndrome 2004: International Registry-diagnosis and management from online to bedside. Hamostaseologie 24:50-55, 2004
7. Ruggeri ZM, Ware J, Ginsburg D: von Willebrand factor, in Loscalzo J, Schafer A (eds): Thrombosis and Hemorrhage. Philadelphia, PA, Lippincott Williams & Wilkins, 2003, pp 246-265
8. Budde U, Schneppenheim R: Von Willebrand factor and von Willebrand disease. Rev Clin Exp Hematol 5:335-368, 2001
9. Titani K, Kumar S, Takio K, Ericsson LH, Wade RD, Ashida K, et al: Amino acid sequence of human von Willebrand factor. Biochemistry 25:3171-3184, 1986
10. Fowler WE, Fretto LJ: Electron microscopy of von Willebrand factor, in Zimmerman TS, Ruggeri ZM (eds): Coagulation and Bleeding Disorders. The Role of Factor VIII and von Willebrand Factor. New York, NY, Dekker, 1989, pp 181-193
11. Mayadas TN, Wagner DD: von Willebrand factor biosynthesis and processing. Ann NY Acad Sci 614:153-166, 1991
12. Katsumi A, Tuley EA, Bodo I, Sadler JE: Localization of disulfide bonds in the cystine knot domain of human von Willebrand factor. J Biol Chem 275:25585-25594, 2000
13. Mayadas TN, Wagner DD: Vicinal cysteines in the prosequence play a role in von Willebrand factor multimer assembly. Proc Natl Acad Sci U S A 89:3531-3535, 1992
14. Haberichter SL, Jozwiak MA, Rosenberg JB, Christopherson PA, Montgomery RR: The von Willebrand factor propeptide (VWFpp) traffics an unrelated protein to storage. Arterioscler Thromb Vasc Biol 22:921-926, 2002
15. Haberichter SL, Merricks EP, Fahs SA, Christopherson PA, Nichols TC, Montgomery RR: Re-establishment of VWF-dependent Weibel-Palade bodies in VWD endothelial cells. Blood Epub ahead of print Aug 26, 2004
16. Cramer EM, Breton-Gorius J, Beesley JE, Martin JF: Ultrastructural demonstration of tubular inclusions coinciding with von Willebrand factor in pig megakaryocytes. Blood 71:1533-1538, 1988
17. Fay PJ, Kawai Y, Wagner DD, Ginsburg D, Bonthron D, Ohlsson-Wilhelm BM, et al: Propolypeptide of von Willebrand factor circulates in blood and is identical to von Willebrand antigen II. Science 232:995-998, 1986
18. Bowie EJ, Solberg LA Jr, Fass DN, Johnson CM, Knutson GJ, Stewart ML, et al: Transplantation of normal bone marrow into a pig with severe von Willebrand's disease. J Clin Invest 78:26-30, 1986
19. Gerritsen HE, Robles R, Lämmle B, Furlan M: Partial amino acid sequence of purified von Willebrnad factor-cleaving protease. Blood 98: 1654-1661, 2001
20. Fujikawa K, Suzuki H, McMullen B, Chung D: Purification of human von Willebrand factor-cleaving protease and its identification as a new member of the metalloproteinase family. Blood 98:1662-1666, 2001
21. Dent JA, Berkowitz SD, Ware J, Kasper CK, Ruggeri ZM: Identification of a cleavage site directing the immunochemical detection of molecular abnormalities in type IIA von Willebrand factor. Proc Natl Acad Sci U S A 87:6306-6310, 1990
22. Dent JA, Galbusera M, Ruggeri ZM: Heterogeneity of plasma von Willebrand factor multimers resulting from proteolysis of the constituent subunit. J Clin Invest 88:774-782, 1991
23. Celikel R, Varughese KI, Madhusudan, Yoshioka A, Ware J, Ruggeri ZM: Crystal structure of von Willebrand factor A1 domain in complex with the function blocking NMC-4 Fab. Nat Struct Biol 5:189-194, 1998
24. Huizinga EG, Tsuji S, Romijn RA, Schiphorst ME, de Groot PG, Sixma JJ, et al: Structures of glycoprotein Ibα and its complex with von Willebrand factor A1 domain. Science 297:1176-1179, 2002
25. Dumas JJ, Kumar R, McDonagh T, Sullivan F, Stahl ML, Somers WS, et al: Crystal structure of the wild-type von Willebrand factor A1-glycoprotein Ibα complex reveals conformation differences with a complex bearing von Willebrand disease mutations. J Biol Chem 279:23327-22334, 2004
26. Ruggeri ZM, Pareti FI, Mannucci PM, Ciavarella N, Zimmerman TS: Heightened interaction between platelets and factor VIII/von Willebrand factor in a new subtype of von Willebrand's disease. N Engl J Med 302:1047-1051, 1980
27. Celikel R, Ruggeri ZM, Varughese KI: von Willebrand factor conformation and adhesive function is modulated by an internalized water molecule. Nat Struct Biol 7:881-884, 2000
28. Goto S, Salomon DR, Ikeda Y, Ruggeri ZM: Characterization of the unique mechanism mediating the shear-dependent binding of soluble von Willebrand factor to platelets. J Biol Chem 270:23352-23361, 1995
29. Siediecki CA, Lestini BJ, Kottke-Marchant K, Eppell SJ, Wilson DL, Marchant RE: Shear-dependent changes in the three-dimensional structure of human von Willebrand factor. Blood 88:2939-2950, 1996
30. Howard MA, Firkin BG: Ristocetin—A new tool in the investigation of platelet aggregation. Thromb Haemost 26:362-369, 1971
31. Brinkhous KM, Read MS, Fricke WA, Wagner RH: Botrocetin (venom coagglutinin): Reaction with a broad spectrum of multimeric forms of factor VIII macromolecular complex. Proc Natl Acad Sci U S A 80: 1463-1466, 1983
32. Fukuda K, Doggett TA, Bankston LA, Cruz MA, Diacovo TG, Liddington RC: Structural basis of von Willebrand factor activation by the snake toxin botrocetin. Structure 10:943-950, 2002
33. Azuma H, Sugimoto M, Ruggeri ZM, Ware J: A role for von Willebrand factor proline residues 702-704 in ristocetin-mediated binding to platelet glycoprotein Ib. Thromb Haemost 69:192-196, 1993
34. Mazzucato M, Spessotto P, Masotti A, De Appollonia L, Cozzi MR, Yoshioka A, Perris R, et al: Identification of domains responsible for von Willebrand factor type VI collagen interaction mediating platelet adhesion under high flow. J Biol Chem 274:3033-3041, 1999
35. Wu D, Vanhoorelbeke K, Cauwenberghs N, Meiring M, Depraetere H, Kotze HF, et al: Inhibition of the von Willebrand (VWF)-collagen interaction by an antihuman VWF monoclonal antibody results in abolition of in vivo arterial platelet thrombus formation in baboons. Blood 99:3623-3628, 2002
36. Ribba AS, Loisel I, Lavergne JM, Juhan-Vague I, Obert B, Cherel G, et al: Ser968Thr mutation within the A3 domain of von Willebrand factor (VWF) in two related patients leads to a defective binding of VWF to collagen. Thromb Haemost 86:848-854, 2001
37. Huizinga EG, van der Plas RM, Kroon J, Sixma JJ, Gros P: Crystal structure of the A3 domain of human von Willebrand factor: Implications for collagen binding. Structure 5:1147-1156, 1997
38. Bienkowska J, Cruz MA, Handin RI, Liddington RC: The von Willebrand factor A3 domain does not contain a metal ion-dependent adhesion site motif. J Biol Chem 272:25162-25167, 1997
39. Romijn RAP, Bouma B, Wuyster W, Gros P, Kroon J, Sixma JJ, et al: Identification of the collagen-binding site of the von Willebrand factor A3-domain. J Biol Chem 276:9985-9991, 2001
40. Romijn RA, Westein E, Bouma B, Schiphorst ME, Sixma JJ, Lenting PJ, et al: Mapping the collagen-binding site in the von Willebrand factor-A3 domain. J Biol Chem 278:15035-15039, 2003
41. Nishida N, Sumikawa H, Sakakura M, Shimba N, Takahashi H, Terasawa H, et al: Collagen-binding mode of vWF-A3 domain determined by a transferred cross-saturation experiment. Nat Struct Biol 10:53-58, 2003

42. Back CH, Radbill JR, Crawford DW: Analysis of pulsatile viscous blood flow through diseased coronary arteries of man. J Biomech 10:339-353, 1977
43. Strony J, Beaudoin A, Brands D, Adelman B: Analysis of shear stress and hemodynamic factors in a model of coronary artery stenosis and thrombosis. Am J Physiol 265:H1787-H1796, 1993
44. Mailhac A, Badimon JJ, Fallon JT, Fernandez-Ortiz A, Meyer B, Chesebro JH, et al: Effect of an eccentric severe stenosis on fibrin(ogen) deposition on severely damaged vessel wall in arterial thrombosis. Relative contribution of fibrin(ogen) and platelets. Circulation 90:988-996, 1994
45. Ruggeri ZM, Mannucci PM, Federici AB, Lombardi R, Zimmerman TS: Multimeric composition of factor VIII/von Willebrand factor following administration of DDAVP: Implications for pathophysiology and therapy of von Willebrand's disease subtypes. Blood 59:1272-1278, 1982
46. Levy GG, Nichols WC, Lian EC, Foroud T, McClintock JN, McGee BM, et al: Mutations in a member of the *ADAMTS* gene family cause thrombotic thrombocytopenic purpura. Nature 413:488-494, 2001
47. Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K: Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. J Biol Chem 276:41059-41063, 2001
48. Moake JL: Thrombotic microangiopathies. N Engl J Med 347:589-600, 2002
49. Ajzenberg N, Denis CV, Veyradier A, Girma J-P, Meyer D, Baruch D: Complete defect in vWF-cleaving protease activity associated with increased shear-induced platelet aggregation in thrombotic microangiopathy. Thromb Haemost 87:808-811, 2002
50. Dong J-F, Moake JL, Nolasco L, Bernardo A, Arceneaux W, Shrimpton CN, et al: ADAMTS-13 rapidly cleaves newly secreted ultra-large von Willebrand factor multimers on the endothelial surface under flowing conditions. Blood 100:4033-4039, 2002
51. Blot E, Deecaudin D, Veyradier A, Bardier A, Zagame O-L, Pouillart P: Cancer-related thrombotic microangiopathy secondary to von Willebrand factor-cleaving protease deficiency. Thromb Res 106:127-130, 2002
52. Xie L, Chesterman CN, Hogg PJ: Control of von Willebrand factor multimer size by thrombospondin-1. J Exp Med 193:1341-1349, 2001
53. Savage B, Almus-Jacobs F, Ruggeri ZM: Specific synergy of multiple substrate-receptor interactions in platelet thrombus formation under flow. Cell 94:657-666, 1998
54. Savage B, Saldivar E, Ruggeri ZM: Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor. Cell 84:289-297, 1996
55. Ruggeri ZM, Bader R, De Marco L: Glanzmann thrombasthenia: Deficient binding of von Willebrand factor to thrombin-stimulated platelets. Proc Natl Acad Sci U S A 79:6038-6041, 1982
56. Ruggeri ZM, De Marco L, Gatti L, Bader R, Montgomery RR: Platelets have more than one binding site for von Willebrand factor. J Clin Invest 72:1-12, 1983
57. Clemetson KJ, Clemetson JM: Platelet collagen receptors. Thromb Haemost 86:189-197, 2001
58. Nieswandt B, Watson SP: Platelet-collagen interaction: is GPVI the central receptor? Blood 102:449-461, 2003
59. Savage B, Sixma JJ, Ruggeri ZM: Functional self-association of von Willebrand factor during platelet adhesion under flow. Proc Natl Acad Sci U S A 99:425-430, 2002
60. Goto S, Sakai H, Goto M, Ono M, Ikeda Y, Handa S, Ruggeri ZM: Enhanced shear-induced platelet aggregation in acute myocardial infarction. Circulation 99:608-613, 1999
61. Ruggeri ZM, Dent JA, Saldivar E: Contribution of distinct adhesive interactions to platelet aggregation in flowing blood. Blood 94:172-178, 1999
62. Zareba W, Pancio G, Moss AJ, Kalaria VG, Marder VJ, Weiss HJ, et al: Increased level of von Willebrand factor is significantly and independently associated with diabetes in postinfarction patients. Thromb Haemost 86:791-799, 2001
63. Theilmeier G, Michiels C, Spaepen E, Vreys I, Collen D, Vermylen J, et al: Endothelial von Willebrand factor recruits platelets to atherosclerosis-prone sites in response to hypercholesterolemia. Blood 99:4486-4493, 2002
64. Methia N, Andre P, Denis CV, Economopoulos M, Wagner DD: Localized reduction of atherosclerosis in von Willebrand factor-deficient mice. Blood 98:1424-1428, 2001
65. Kasirer-Friede A, Ware J, Leng L, Marchese P, Ruggeri ZM, Shattil SJ: Lateral clustering of platelet GP Ib-IX complexes leads to up-regulation of the adhesive function of integrin $\alpha_{IIb}\beta_3$. J Biol Chem 277:11949-11956, 2002
66. Kasirer-Friede A, Cozzi MR, Mazzucato M, De Marco L, Ruggeri ZM, Shattil SJ: Signaling through GP Ib-IX-V activates αIIbβ3 independently of other receptors. Blood 103:3403-3411, 2004
67. Mazzucato M, Pradella P, Cozzi MR, De Marco L, Ruggeri ZM: Sequential cytoplasmic calcium signals in a two-stage platelet activation process induced by the glycoprotein Ibα mechanoreceptor. Blood 100: 2793-2800, 2002
68. Yap CL, Anderson KE, Hughan SC, Dopheide SM, Salem HH, Jackson SP: Essential role for phosphoinositide 3-kinase in shear-dependent signaling between platelet glycoprotein Ib/V/IX and integrin $\alpha_{IIb}\beta_3$. Blood 99: 151-158, 2002
69. Mazzucato M, Cozzi MR, Pradella P, Ruggeri ZM, De Marco L: Distinct roles of two ADP receptors in von Willebrand factor-mediated platelet signaling and activation under flow. Blood Epub ahead of print July 29, 2004
70. Falati S, Edmead CE, Poole AW: Glycoprotein Ib-V-IX, a receptor for von Willebrand factor, couples physically and functionally to the Fc receptor γ-chain, Fyn, and Lyn to activate human platelets. Blood 94: 1648-1656, 1999
71. Torti M, Bertoni A, Canobbio I, Sinigaglia F, Lapetina EG, Balduini C: Rap 1B and Rap2B translocation to the cytoskeleton by von Willebrand factor involves FcgammaII receptor-mediated protein tyrosine phosphorylation. J Biol Chem 274:13690-13697, 1999
72. Wu Y, Suzuki-Inoue K, Satoh K, Asazuma N, Yatomi Y, Berndt MC, et al: Role of Fc receptor gamma-chain in platelet glycoprotein Ib-mediated signaling. Blood 97:3836-3845, 2001
73. Sullam PM, Hyun W, Szollosi J, Foss WM, Lopez JA: Physical proximity and functional interplay of the glycoprotein Ib-IX-V complex and the Fc receptor FcΨRIIa on the platelet plasma membrane. J Biol Chem 273:5331-5336, 1998
74. Lefkovits J, Plow EF, Topol EJ: Platelet glycoprotein IIb/IIIa receptors in cardiovascular medicine. N Engl J Med 332:1553-1559, 1995
75. Goto S, Ikeda Y, Saldivar E, Ruggeri ZM: Distinct mechanisms of platelet aggregation as a consequence of different shearing flow conditions. J Clin Invest 101:479-486, 1998
76. Ni H, Subbarao S, Denis C, Degen JL, Hynes RO, Wagner DD: Distinct roles of von Willebrand factor and fibrinogen in thrombus growth in arterioles. Thromb Haemost 82:411, 1999 (suppl, abstr)
77. Ni H, Denis CV, Subbarao S, Degen JL, Sato TN, Hynes RO, Wagner DD: Persistence of platelet thrombus formation in arterioles of mice lacking both von Willebrand factor and fibrinogen. J Clin Invest 106: 385-392, 2000
78. Ni H, Yuen PS, Papalia JM, Trevithick JE, Sakai T, Fassler R, et al: Plasma fibronectin promotes thrombus growth and stability in injured arterioles. Proc Natl Acad Sci U S A 100:2415-2419, 2003
79. Andre P, Prasad KS, Denis CV, He M, Papalia JM, Hynes RO, et al: CD40L stabilizes arterial thrombi by a β_3 integrin-dependent mechanism. Nat Med 8:247-252, 2002