

Von Willebrand Disease and the Mechanisms of Platelet Function

Zaverio M. Ruggeri

Von Willebrand Factor and von Willebrand Disease

The study of patients with von Willebrand disease has prompted rapid progress in the understanding of the structure and function of von Willebrand factor (vWF), a protein with a central role in the complex process of platelet thrombus formation. Von Willebrand disease was first described in 1926.^(1,2) It is the most common inherited disorder of hemostasis, with a prevalence as high as 0.83%,⁽³⁾ and is characterized by a complex hemostatic defect. Abnormal platelet function, expressed by prolonged bleeding time, is a consistent finding and may be accompanied by decreased factor VIII procoagulant activity. The pathogenesis of von Willebrand disease is based on quantitative and/or qualitative abnormalities of vWF, a large multimeric glycoprotein with two distinct biological roles: it mediates platelet adhesion and thrombus formation at sites of vascular injury; and it serves as the carrier for procoagulant factor VIII in circulating blood, where the two molecules are present as the factor VIII/vWF complex.^(4,5) Mature vWF has a typical multimeric structure and exists as a series of oligomers containing a variable number of subunits. Individual multimers range in mass from approximately 500 kDa to > 10,000 kDa, the latter being the largest known for a soluble human plasma protein. The mature vWF subunit contains 2,050 amino acid residues and up to 22 carbohydrate side chains.

The vWF gene, consisting of ~180 kilobases and containing 52 exons,⁽⁶⁾ is located at the tip of the short arm of chromosome 12, region 12p12-12pter.⁽⁷⁾ A non-processed vWF pseudogene has been identified on chromosome 22.⁽⁸⁾ The primary translation product predicted from the cloned vWF cDNA is a 2,813-residue precursor polypeptide referred to as pre-pro-vWF⁽⁹⁾; it consists of a 22-residue signal peptide, an unusually large 741-residue propeptide and the mature subunit of 2,050 residues. The propeptide and mature subunit of vWF are almost entirely composed of four types of repeating domains, designated A through D.⁽¹⁰⁾ The normal synthesis of vWF occurs in endothelial cells⁽¹¹⁾ and megakaryocytes.⁽¹²⁾ Following translation of the mRNA, pro-vWF undergoes extensive post-translational processing to produce multimeric vWF.^(13,14) The vWF propeptide is removed from the mature protein and circulates as a distinct molecule previously identified and characterized as von Willebrand antigen II.^(15,16)

Von Willebrand disease exhibits significant phenotypic heterogeneity. Two main categories of patients can be identified, depending on whether the pathogenesis is based on quantitative (type 1 and type 3) or qualitative (type 2) defects of vWF. The classification of von Willebrand disease has been recently revised by the Standardization Committee of the International Society of Thrombosis and Hemostasis.⁽¹⁷⁾

Type 1 is the most common form of the disease, accounting for approximately 70% of all cases. It is inherited as an autosomal dominant, mild to moderately severe bleeding disorder. The disease, in these cases, is due to inadequate levels of vWF in plasma, always accompanied by a parallel decrease in factor VIII procoagulant activity.

Thus, although both vWF and factor VIII are structurally and functionally intact in type 1 patients, the decreased concentration, usually between 5% and 30% of normal, causes impaired function. Little is known, to date, about the molecular pathogenesis of type 1 von Willebrand disease.

Type 3 von Willebrand disease, transmitted as an autosomal recessive bleeding diathesis with severe to very severe manifestations, is the least common of all forms of von Willebrand disease, with an incidence of approximately 1 in 1,000,000 subjects. Type 3, like type 1 von Willebrand disease, is due to a quantitative defect of vWF, but the relative severity of the two forms is clearly different since the levels of plasma vWF in the former type are usually undetectable even with very sensitive assays. Abnormalities of the vWF gene have been detected in several families with type 3 von Willebrand disease. These abnormalities vary from deletions of various size (from as small as 2.3 kilobase to the entire 178 kilobase gene) to single nonsense mutations.⁽¹⁸⁾ Moreover, there is also evidence for the occurrence of *cis*-acting mutations affecting vWF mRNA transcription, processing or stability.⁽¹⁹⁾

Type 2 von Willebrand disease comprises many different subtypes and is phenotypically heterogeneous.⁽²⁰⁾ Common to all subtypes is the occurrence of qualitative abnormalities of vWF resulting, in most cases, in abnormal multimeric structure of the molecule.^(21,22) The disease, in these cases, is due to the existence of functional defects of vWF that result in impaired platelet function even though the plasma concentration of vWF (as well as factor VIII procoagulant) may be only modestly reduced or even normal. Single point missense mutations of the vWF gene have been identified in a number of patients, mainly with the two relatively frequent type 2A and type 2B von Willebrand disease.⁽²³⁾

Platelets and Shear Forces

Platelet Adhesion to Thrombogenic Surfaces

The first step in the response of platelets to vascular injury is their irreversible attachment to the site of lesion exposed to flowing blood. This process is influenced by an essential aspect of the regulation of platelet function, namely the ability to react to surfaces that are different from the normal vessel wall. Two possible mechanisms can be envisioned in this regard. Substances (agonists) generated as a consequence of the lesion may “activate” platelets and lead to their interaction with adhesive molecules in blood or exposed at the site of injury. Alternatively, or in addition, circulating “resting” platelets may react with adhesive substrates exposed only where the vessel wall is altered. A combination of both events is likely to occur during the formation of platelet thrombi; however, the response of “resting” platelets to a local adhesive environment created by the lesion in the vessel wall probably represents the very initial response onto which the subsequent formation of a hemostatic plug is built.

Platelet Aggregation

The process leading to a thrombus capable of arresting hemorrhage involves the interaction of platelets with one another. This occurs after the initial adhesion of platelets onto a thrombogenic substrate at the site of injury, and can actually be visualized as

adhesion occurring onto already adhering platelets, or cohesion of platelets. Like adhesion, it is likely to involve both the recognition of activated adhering platelets as an appropriate adhesive substrate by “resting” circulating platelets, as well as the action of local agonists that “activate” platelets. Of note, a significant proportion of the current concepts on the mechanisms of platelet aggregation are based on experimental models that study platelets in suspension in the absence of a reactive surface; however, aggregation preceding adhesion may never occur during hemostasis *in vivo*.

Blood Viscosity, Shear Stress and Platelet Function

The role of platelets in hemostasis and thrombosis requires that they become irreversibly attached at a site of injury. This occurs against the tendency of flow to move adhering platelets with the layer of blood adjacent to the surface. Shear stress results from differences in the velocity of blood, which is minimal near the vessel wall and increases progressively towards the center of the vascular lumen. The force opposing stable adhesion and aggregation is greater with increasing shear stress; consequently, it can be surmised that shear-dependent phenomena are of particular relevance in those districts of the vasculature where shear forces are greater, i.e., in arteries more than in veins and, particularly, in arterioles.⁽²⁴⁾ Moreover, in the presence of partial obstructions of the vessel lumen, as may be caused by atherosclerotic plaques and/or vasospasm, fluid shear stress may increase considerably above the time average level of about 20 dynes/cm² calculated for the circulation of blood in normal vessels. The influence of shear-dependent phenomena, therefore, may be greater in pathological conditions predisposing to the occurrence of acute arterial occlusion than it is in the course of normal hemostasis.

Effect of Shear Stress on Platelet Aggregation: The Role of von Willebrand Factor

Normal platelets aggregate when stimulated by an appropriate agonist. Those that may be present at a site of vascular injury include thrombin, collagen and ADP, but none is present in relevant amounts in circulating blood. Alternatively, aggregation occurs when platelets are exposed to shear stress above 60-80 dynes/cm². In the latter case, “activation” depends on mechanisms at least in part distinct from those responsible for aggregation induced by exogenous chemical agonists. A specific platelet receptor, the membrane glycoprotein (GP) Ib-IX-V complex, and the adhesive protein that binds to it, vWF, are necessary for aggregation induced by high shear^(25,26) but not for agonist-induced aggregation. Moreover, it is now established that the adhesive protein mediating aggregation through the integrin receptor $\alpha_{IIb}\beta_3$ (the GP IIb-IIIa complex) is different in a low shear environment as opposed to a high shear one: fibrinogen is involved in the first instance, vWF in the second.

Hypotheses have been formulated to explain how shear stress can induce aggregation of platelets in suspension.^(27,28) It is believed that the binding of multimeric vWF to GP Ib with shear forces above 60-80 dynes/cm² causes a transmembrane flux of calcium ions increasing their intracellular concentration by two- to threefold. This results in activation of platelets, not dissimilar from that induced by chemical agonists. Activation of platelets, in turn, confers to another receptor, $\alpha_{IIb}\beta_3$, the ability to interact with soluble adhesive proteins and, thus, mediate platelet-to-platelet interaction

(aggregation). Shear-induced aggregation depends on vWF both for its initiation, to activate platelets as other agonists can do in lower shear environments, and for its completion, to support platelet cohesion. This dual function is rather unique and is supported by two distinct sites in the molecule and two different receptors.^(28,29) Inhibition of the shear-induced vWF-GP Ib interaction results in the suppression of both the transmembrane calcium flux and aggregation; inhibition of vWF binding to $\alpha\text{IIb}\beta\text{3}$ interferes only with aggregation, not with the calcium ion flux.⁽²⁸⁾ These findings establish the temporal sequence of events as “activation” and “cohesion,” both vWF dependent under high shear conditions. The regulation of these events was thought to depend on the modulation of vWF affinity for GP Iba. Recent studies, however, have demonstrated that vWF binding to platelets under shear, not only aggregation, is a dual receptor process, requiring both GP Iba and $\alpha\text{IIb}\beta\text{3}$, and may be regulated by mechanisms that control platelet activation.⁽³⁰⁾

Function of von Willebrand Factor in Platelet-Surface and Platelet-Platelet Interactions

The local adhesion of platelets recruited from the circulation represents the initial event of thrombus formation on a surface and is followed by accumulation of additional platelets onto the adhering ones. The latter process requires the interaction of soluble ligands with activated $\alpha\text{IIb}\beta\text{3}$. This platelet receptor exhibits distinct specificity for different ligands depending on whether the latter are immobilized or in solution. When platelets are activated, $\alpha\text{IIb}\beta\text{3}$ is a promiscuous binding site, capable of interacting with at least four different ligands (fibrinogen, vWF, fibronectin and vitronectin). In remarkable contrast, $\alpha\text{IIb}\beta\text{3}$ on nonactivated platelets shows the ability to interact only with immobilized fibrinogen.^(29,31) It is apparent, therefore, that GP Ib and $\alpha\text{IIb}\beta\text{3}$ represent two pathways by which nonstimulated platelets can attach to a thrombogenic surface that presents fibrinogen (fibrin) and/or vWF exposed to flowing blood. There is, however, a fundamental difference between the processes initiated by the two different adhesive proteins. Irreversible adhesion to fibrinogen occurs even when platelets are metabolically inhibited, a condition that does not allow subsequent aggregation. In contrast, when platelets are metabolically inhibited there is no irreversible adhesion onto a vWF-coated surface, in spite of a normal initial contact with this adhesive protein. Recent studies have demonstrated that GP Iba supports the continuous translocation of platelets interacting with immobilized vWF.⁽³²⁾ This, in itself, does not lead to irreversible arrest onto the surface but is a necessary prerequisite for adhesion at high shear rates. In fact, only platelets that are kept in continuous contact with the surface, even though in slow motion, can become permanently attached through an $\alpha\text{IIb}\beta\text{3}$ -mediated mechanism regardless of hemodynamic conditions; in contrast, $\alpha\text{IIb}\beta\text{3}$ by itself can directly mediate arrest of platelets but only at relatively low shear rates.⁽³²⁾

On the basis of the experimental results presented above, it is possible to delineate an initial understanding of the mechanisms of platelet function that allow thrombus propagation initiated and mediated by adhesive components at the site of vessel injury. Thus, the recognition of an appropriate surface-bound adhesive protein represents the first step in the process of thrombus formation. This platelet-surface contact must be followed

by platelet spreading, necessary to make the initial contact irreversible (adhesion), and by activation, necessary to lead to cohesion of additional platelets into the forming thrombus. Spreading requires interaction of the surface-bound ligands with $\alpha_{IIb}\beta_3$. In the case of fibrinogen (fibrin) it can proceed even if platelets are not fully activated since the receptor possesses the appropriate recognition specificity already expressed on resting platelets.⁽³³⁾ In the case of vWF, however, full platelet activation is necessary for $\alpha_{IIb}\beta_3$ to be able to recognize the ligand and mediate spreading. Adhesion receptors clearly have signaling function, in addition to supporting mechanically the attachment of platelets to a surface and to one another. On the other hand, immobilized vWF and fibrinogen (fibrin) act as proper platelet agonists, in addition to mediating adhesive events.

The shear stress in flowing blood has profound effects on the processes that lead to thrombus formation since, as delineated above, the pathways mediated by vWF and GP Ib acquire predominant relevance with increasing shear rate. In fact, thrombus formation onto a type I collagen surface is independent of vWF up to a shear rate of 800 s⁻¹, but becomes largely dependent on vWF with increasing shear rate.⁽³⁴⁾ As mentioned above, extreme values of shear stress never attained in the normal circulation (and, presumably, not of concern for the normal hemostatic processes) are reached under pathological conditions in stenosed atherosclerotic vessels. Thus, it is possible that selective pharmacological intervention aimed at blocking vWF binding to GP Ib (the interaction that initiates vWF-dependent mechanisms of platelet adhesion and aggregation) may result in effective anti-thrombotic therapy with lesser hemorrhagic side effects. A detailed knowledge of how flow-related parameters influence the process of thrombus formation may, therefore, provide the means to understand fully the mechanism of normal hemostasis and to prevent pathological thrombosis.

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