



Review Article

On the Versatility of von Willebrand Factor

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Abstract. Von Willebrand factor (VWF) is a large multimeric protein, the function of which has been demonstrated to be pivotal to the haemostatic system. Indeed, quantitative and/or qualitative abnormalities of VWF are associated with the bleeding disorder Von Willebrand disease (VWD). Moreover, increased plasma concentrations of VWF have been linked to an increased risk for thrombotic complications. In the previous decades, many studies have contributed to our understanding of how VWF is connected to the haemostatic system, particularly with regard to structure-function relationships. Interactive sites for important ligands of VWF (such as factor VIII, collagen, glycoprotein Iba, integrin α IIB β 3 and protease ADAMTS13) have been identified, and mutagenesis studies have confirmed the physiological relevance of the interactions between VWF and these ligands. However, we have also become aware that VWF has a more versatile character than previously thought, given its potential role in various non-hemostatic processes, like intimal thickening, tumor cell apoptosis and inflammatory processes. In the present review, a summary of our knowledge on VWF structure-function relationships is provided in the context of the "classical" haemostatic task of VWF and in perspective of pathological processes beyond haemostasis.

Introduction. Von Willebrand factor (VWF) is a protein that has historically been known for its role in the haemostatic process. However, many hurdles had to be overcome between the initial description in 1926 of the bleeding tendency that is now known as Von Willebrand disease (VWD)¹ and the identification of the protein that is associated with this disorder. Indeed,

it took 30 years after the seminal paper by Erik von Willebrand, before it was reported that the bleeding episodes in von Willebrand disease could be corrected upon the infusion of a plasma factor.² The search for the identity of this plasma component was far from simple. Indeed, it was complicated by the multimeric nature of VWF and the notion that VWF circulates in

complex with coagulation factor VIII (FVIII), the protein that is associated with hemophilia A. The technical difficulties that needed to be addressed have nicely been put in context in several personal anecdotes describing the events that led to the discovery in the early 1970s that VWF and FVIII are separate proteins and that VWF is a multimeric protein.³⁻⁵ The identification of VWF as a plasma component that is associated with VWD provided the basis for numerous additional studies. For starters, the purified protein was used to determine its sequence, which in turn was needed to clone the gene encoding VWF.⁶⁻¹⁰ This breakthrough stimulated the rapid expansion of our knowledge on the epidemiology, genetics and molecular basis of VWD.^{11,12} With the help of recent multicenter studies in Europe, Canada and the USA, our insight into the complex genetic background of VWD has been dramatically improved, a necessary step to further refine clinical and laboratory diagnosis of the disease.^{13,14} Clinical studies further taught us that the critical role of VWF in haemostasis is not only obvious from the bleeding tendency that is associated with its functional deficiency, but also in view of its relationship with thrombotic disorders. Increased levels of VWF have been shown to be predictive for atherothrombotic complications.¹⁵⁻²² In line with VWF being a risk factor for atherothrombotic complication, a recent study reported a reduced prevalence of arterial thrombosis in patients with VWD.²³ Importantly, the

role of VWF in the development of thrombotic complications is not limited to myocardial infarctions, but also include stroke and venous thrombosis.²⁴⁻²⁸ Of note, the contribution of VWF to venous thrombosis may be both direct as part of a complex with neutrophil extracellular traps (NETs),²⁹ and indirect via FVIII (which is an independent risk factor for venous thrombosis), given the role of VWF as a determinant of FVIII plasma levels.^{30,31}

Fundamental studies on the structure-function relationships of VWF provided insight into how this multimeric protein supports the different aspects of the haemostatic process. Importantly, from these studies it also became clear that VWF has a more versatile character than previously thought, given its potential role in various non-hemostatic processes, like cell proliferation and tumor cell apoptosis.³² In the present review, an overview of our current knowledge of VWF structure and function will be provided. Subsequently, we will describe the contribution of VWF to (patho)-physiological processes beyond haemostasis. Finally, we will discuss how shear stress and modulation of multimer size regulate classical and novel functions of VWF.

Structure of VWF. The biosynthesis of VWF has been described extensively in several excellent reviews (see for example Wagner³³ and Sadler^{34,35}). Its synthesis is limited to endothelial cells and megakaryocytes,^{36,37}

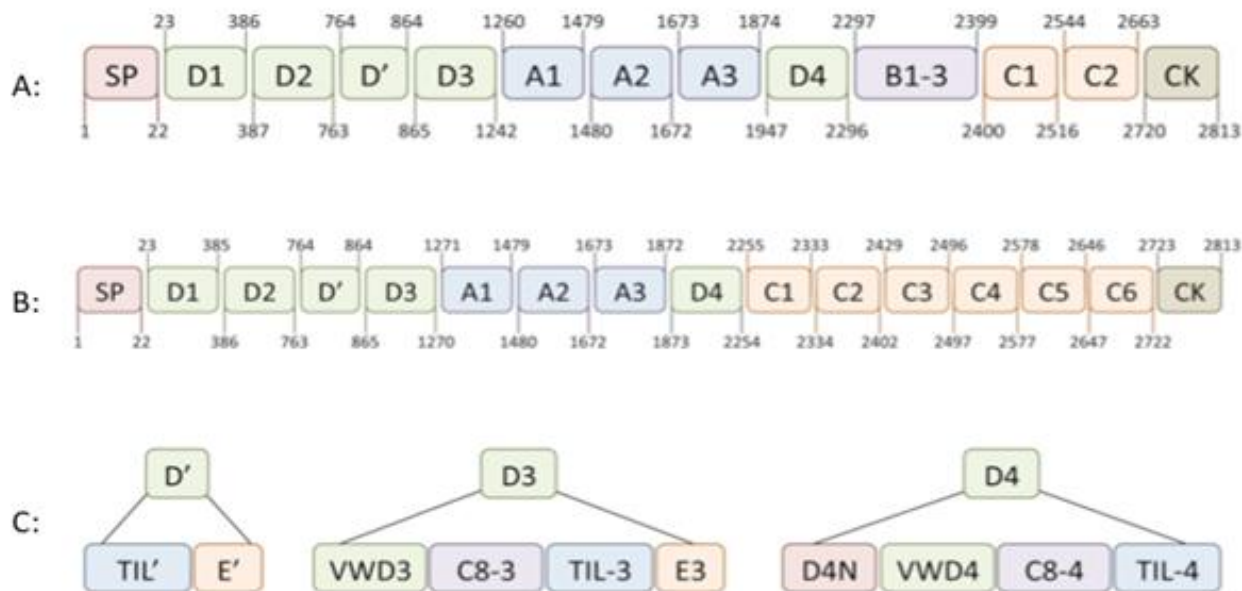


Figure 1. Domain structure of VWF. The molecular architecture of VWF is characterized by the presence of distinct domain structures. Panel A represents the arrangement of five different structures according to the original analysis of the VWF sequence (reviewed by Pannekoek & Voorberg).³⁸ The numbering of the domain boundaries has been used in our laboratory in the previous years. Panel B shows the domain organization as has recently been proposed by Zhou et al.⁴¹ One striking difference with the original domain structure is the replacement of the B1-3 - C1 - C2 domain region by 6 homologous C-domains. In addition, their analysis revealed that the D-domains consist of various independent structures, which are highlighted in panel C. The D1, D2 and D3 domains each contain a VW-domain, a trypsin inhibitor-like (TIL)-structure, a C8 fold and an E module. The D' region lacks the VW domain and TIL-structure. The D4 domain lacks the E module, but instead comprises a unique sequence designated D4N.

where it is produced as a single chain pre-pro-protein. It consists of a 22-amino acid signal peptide, a 741-amino acid propeptide and a mature subunit of 2050 amino acids (**Figure 1**).³³⁻³⁵ Initial analysis of the VWF primary structure revealed that the molecular architecture of the pro-protein distinguishes a discrete domain structure, arranged as D1-D2-D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK, with the propeptide comprising the D1-D2 domains, and the mature subunits the remaining domains.^{33-35,38} More recently, the domain structure of VWF has been re-evaluated using structural information from other proteins with homologous domains in combination with electron microscopy techniques.³⁹⁻⁴¹ This exercise revealed a number of interesting insights. First, it allowed a more detailed assignment of disulfide bonds between cysteines throughout the molecule.⁴¹ Second, it appeared that the D-domains have a more complex structure than initially thought. In fact, D-domains consist of 4 independent structures: a von Willebrand domain, a Cysteine-8 structure, a trypsin-inhibitor-like (TIL)-fold and an E module (**Figure 1**).⁴¹ Third, the region carboxyterminal to the D4 domain (i.e. the B1-B2-B3-C1-C2 domains) was recognized to consist of six consecutive C-domains instead of two, with the Arg-Gly-Asp (RGD)-integrin recognition sequence being located in the C4 domain (**Figure 1**).⁴¹ These new insights in VWF structure will help us to better understand the cross-talk between domains in the functions of VWF.

A most intriguing aspect of VWF biology concerns the multimeric structure of the protein. The mature VWF protein exists as a heterologous series of covalently-linked mature subunits ranging from dimers (molecular weight 0.5 millionDa) to large polymers consisting of over 40 subunits (molecular weight >20 millionDa).³³⁻³⁵ As will be discussed later in this review, the multimer structure is important for a subset of VWF functions, and regulation of multimer size and quaternary structure is an important tool to modulate these functions.

The classical functions of VWF: FVIII binding. The intricate linkage between VWF and FVIII is perhaps best illustrated by the nomenclature that was previously used to distinguish between the coagulation- and platelet-related activities of the complex: FVIII coagulant activity (FVIII:C), FVIII related antigen (FVIII:RAg) and FVIII ristocetin cofactor activity (FVIII:RCF).⁴² In fact, some still use the term FVIII:RAg instead of VWF to describe staining of endothelial cells in the immunohistochemical analysis of healthy and pathological tissues (see *e.g.* Bauer *et al.*⁴³).

VWF and FVIII circulate in a tight non-covalent

complex in the circulation, and the affinity is estimated to be less than 1 nM.^{44,45} The binding site for FVIII is located in the amino-terminal D'D3 region, spanning residues 764-1035.^{46,47} In a recent study, Castro-Nunez and coworkers used an approach of mass spectrometer-assisted footprinting to discover that VWF residues Ser-764 and Lys-773 seem to be directly involved in the binding of FVIII.⁴⁸ The complementary binding site in FVIII has also been identified, involving residues at both the amino- and carboxyterminal regions of the FVIII light chain.^{49,50}

The physiological relevance of VWF/FVIII complex formation is exemplified by the markedly reduced FVIII plasma levels in patients with undetectable VWF levels (VWD-type 3) or with a defect in the FVIII-interactive site of VWF (VWD-type 2N).⁵¹⁻⁵³ Indeed, the majority of VWD-type 2N mutations are located in the region spanning residues 764-1035,⁵⁴ suggesting that these mutations affect FVIII binding directly by modulation of the FVIII interactive site.

VWF protective function towards FVIII is related to several aspects:⁵⁵ (1) VWF stabilizes the heterodimeric structure of FVIII;⁵⁶ (2) VWF protects FVIII from proteolytic degradation by phospholipid-binding proteases like activated protein C and activated factor X (FXa);^{57,58} (3) VWF interferes with binding of FVIII to negatively-charged phospholipid surfaces, which are for example exposed on activated platelets;⁴⁵ (4) VWF inhibits binding of FVIII to activated factor IX (FIXa), thereby denying FVIII access to the FX-activating complex;⁵⁹ (5) VWF shields FVIII from part of the inhibitory antibodies that may be generated during FVIII-replacement therapy in about 30% of the severe hemophilia A patients;⁶⁰⁻⁶³ and (6) VWF prevents the uptake of FVIII by some cells, including dendritic cells.^{64,65} In view of the role of dendritic cells in antigen-presentation to T-cells, this latter function may be of relevance regarding the immune-response towards FVIII that has been observed in the treatment of hemophilia patients. In several *in vivo* studies using mice, it has been shown that the addition of VWF reduces the immune-response towards FVIII.^{63,66-68} This may suggest that the presence of VWF in therapeutic FVIII preparations may influence the development of inhibitory antibodies, although epidemiological studies have revealed conflicting data on this possibility.⁶⁹⁻⁷³

Apart from its protective role, VWF may also play a role in the targeting of FVIII to sites of vascular injury.⁷⁴ It should be noted that complex formation is not an absolute requirement for FVIII to reach the developing thrombus, as has been shown in studies using VWF-deficient mice.^{75,76}

The classical functions of VWF: collagen binding.

Shortly after the identification of VWF as a plasma protein, its capacity to adsorb to collagens was reported.⁷⁷ Subsequent studies revealed that a dominant binding site for collagen in VWF is located in the VWF A3 domain involving a discontinuous epitope.⁷⁸⁻⁸¹ The A3 domain is able to interact with various types of collagen, including collagens I & III, and the complementary binding sequences in collagen I and III have been deciphered in detail.⁸²⁻⁸⁵ The importance of the A3 domain in binding to collagen is supported by the finding that mutations in or around the collagen binding site may be associated with an increased bleeding tendency.⁸⁶⁻⁸⁹

An alternative binding-site for collagen in the VWF protein is located in the A1 domain, as has been demonstrated by various research groups.^{83,90-93} However, opposite findings have been reported concerning the contribution of the A1-domain in facilitating VWF-platelet interactions under conditions of flow.^{79,94} Nevertheless, it appears that some mutations in the A1 domain found in VWD patients may affect collagen binding, providing a rationale for the bleeding tendency in these patients.⁹⁵⁻⁹⁸

It should be noted that the bleeding tendency associated with mutations in the collagen binding site is usually mild, which is in line with the observation that a murine VWF variant with a defective collagen binding in the A3 domain displays no defect in the correction of the bleeding time in a tail clip-model for normal haemostasis.^{99,100} In contrast, this mutant shows a strongly delayed occlusion time in a ferric chloride-induced model of vascular injury, suggesting that blocking VWF-collagen interactions could be a potential therapeutic approach in the treatment of arterial thrombosis.¹⁰⁰ This possibility has been explored in animal models for thrombosis, revealing that antibodies blocking VWF-collagen interactions are efficient in reducing the thrombotic tendency.^{101,102}

Many *in vitro* studies revealed that VWF-collagen interactions are needed for the recruitment of platelets particularly under conditions of high shear rates (for reviews see Sixma *et al.*¹⁰³ and Nuyttens *et al.*¹⁰⁴). In spite of this important function, defects in collagen binding are associated with only a mild bleeding tendency. The explanation for this apparent contradiction may originate from the redundancy in the process that mediates platelets adheres to collagen. First, VWF contains multiple collagen-binding sites, which may perhaps compensate for each other under particular conditions. Second, platelets contain other collagen receptors, such as $\alpha 2\beta 1$ and Glycoprotein-VI (GpVI), that could allow them to interact with collagen in the absence of VWF.¹⁰⁵ It should be mentioned that both receptors do not resist high shear forces,¹⁰⁵

indicating that they are unable to function as a back-up system for VWF under high shear conditions. Finally, the subendothelial matrix comprises also other components that function as an adhesive surface for VWF, such as tenascin-C and laminin.^{106,107} However, the binding sites for these proteins have not yet been identified.

From a structural point of view, the multimeric VWF protein attached to the collagen surface will undergo shear stress-induced conformational changes that lead to the exposure of the binding site for its platelet-receptor GpIb.¹⁰⁸ Interestingly, binding to collagen has also a secondary effect, in that it results in release of FVIII from the VWF molecule. This phenomenon was already recognized in the original manuscript that described the adsorption of VWF to collagen,⁷⁷ and was further elaborated by Bendetowicz and colleagues.¹⁰⁹ The reason for this release is yet unclear, but it could be that release of FVIII from collagen-bound VWF makes it more rapidly available for the coagulation cascade: VWF-bound FVIII is poorly activated by FXa/phospholipids, whereas VWF-free FVIII is efficiently activated by this complex.⁵⁷ Alternatively, this collagen-induced release could be a mechanism to prevent FVIII binding to VWF that is located in the subendothelial matrix, preventing undesired extravasation of FVIII.¹⁰⁹

The classical functions of VWF: platelet binding.

A key function of VWF is to mediate the recruitment of platelets to sites of vascular injury, especially at those locations where collagen-binding platelet-receptors do not resist high shear forces. Interactions between platelets and VWF are mediated by two distinct platelet receptors: GpIb α and integrin α IIB β 3. GpIb α is part of the GpIb-IX-V complex that is abundantly expressed at the platelet surface.^{110,111} Contacts between VWF and GpIb α require the VWF A1 domain, and the GpIb α interactive site has been elucidated at the atomic level using co-crystal structures of the VWF A1 domain and a soluble GpIb α fragment.¹¹²⁻¹¹⁴ Mutations of residues in the VWF A1 domain that cover the interactive surface with GpIb α have indeed found to be associated with impaired VWF function and a bleeding tendency in patients with VWD-type 2M.¹² The VWF-GpIb α interaction is probably the best-studied aspect of VWF at both the functional and structural level, and its importance for the formation of platelet-rich thrombi has been extensively reviewed elsewhere (see for instance references^{108,115-117}).

The binding site for α IIB β 3 is located in the C1 domain of VWF (C4 domain according to the new annotation proposed by Zhou *et al.*⁴¹) and involves the classical Arg-Gly-Asp (RGD) recognition sequence for integrins. The function of the VWF- α IIB β 3 interaction

is related to the enforcement of platelet-platelet interactions as has been demonstrated in several *in vitro* studies.¹¹⁸⁻¹²¹ However, since several other ligands (notably fibrinogen) are capable of doing so as well, this VWF function has long been thought to be redundant. This view is compatible with the notion that so far no patients having mutations in the α Ib β 3 binding sequence have been reported. However, studies using a mouse model expressing a VWF mutant with defective α Ib β 3 binding have forced us to change this view. Although mice expressing this mutant show normal correction of the bleeding time in a tail clip-model for haemostasis, they are characterized by an impaired vessel occlusion time in a ferric chloride-induced model of vascular injury.^{99,100} A similar reduction of vessel occlusion was observed in mice treated with antibodies against the RGD-sequence of VWF.¹⁰² More detailed analysis of thrombus formation in these mice revealed that initial thrombus formation is unaffected. However, larger thrombi seem to dissolve as a result of the increased hydrodynamic forces to which the growing thrombus is exposed.^{100,102} This strongly suggests that the VWF- α Ib β 3 interaction is not redundant, but of physiological relevance with regard to the stabilization of the growing thrombus.

Novel aspects of VWF function: the molecular bus.

As described in the paragraph “The classical functions of VWF: FVIII binding”, VWF is particularly known as a carrier protein for FVIII in the circulation to maintain appropriate FVIII plasma levels. However, in recent years it has become clear that FVIII is not the only protein that circulates in complex with VWF in the circulation. Other examples of proteins that are associated with VWF in plasma include ADAMTS13,^{122,123} osteoprotegerin,¹²⁴⁻¹²⁶ angiopoietin-2 (Christophe OD, Cherel G, Lenting PJ, Denis CV; unpublished publications) and two members of the galectin family, galectin-1 and galectin-3.¹²⁷ It would not be surprising if this list of VWF-bound proteins will grow in the future. For instance, Turner & Moake recently published that several members of the complement family (*i.e.* C3, C5 and factors B, D, P H & I) attach to VWF that is freshly released from endothelial cells.¹²⁸ It seems reasonable to assume that at least some of these proteins remain associated to VWF upon release from the endothelial surface into the circulation.

Like for FVIII, galectin-1 and galectin-3 plasma levels were higher in wild-type mice compared to mice deficient for VWF,^{127,129} suggesting that VWF is needed to stabilize galectin-1 and galectin-3 in the circulation. With regard to osteoprotegerin, a recent study revealed a positive correlation between VWF and osteoprotegerin levels in a cohort consisting of patients

with cardiovascular disease and asymptomatic controls.¹²⁶ This correlation appeared particularly relevant in asymptomatic individuals without coronary calcification. These recent findings might suggest that VWF could play a similar protective role to stabilize osteoprotegerin in plasma. Of course, additional studies are needed to support this point of view. An opposite observation has been made regarding ADAMTS13 in that an inverse relationship between plasma levels of VWF and ADAMTS13 was reported.¹³⁰ In addition, ADAMTS13 levels were ~40% higher in patients lacking circulating VWF than in control individuals.¹³⁰ How VWF influences ADAMTS13 plasma levels remains to be determined. One possible explanation can be that VWF-bound ADAMTS13 is cleared in *conjunction* with VWF, which has a shorter half-life than ADAMTS13.^{131,132}

The wide variety of proteins that are bound to VWF in the circulation raises a number of questions. First, how many passengers can be on the VWF bus at the same time? For FVIII and both galectins, we know that their plasma concentrations are about 100-fold lower than that of VWF, which suggests that they will not occupy all the places that are available. As for ADAMTS13, Feys *et al.* calculated that it circulates in complex with VWF in a stoichiometry of 1:250, also indicating that the majority of the VWF subunits remain non-occupied.¹²³ A second question is: what are the functional consequences of complex formation? VWF protects FVIII and may promote its targeting to sites of vascular injury. In contrast, FVIII may have the opposite effect on VWF, as it has been reported that the presence of FVIII promotes VWF degradation by ADAMTS13.¹³³ With regard to the galectins, angiopoietin-2 and osteoprotegerin, the functional consequences of their binding to VWF have been investigated to a limited extent, if at all. In view of the large size of the VWF protein, it seems conceivable that VWF has a profound effect of the functionality of these proteins in that it may prevent the interaction with their natural ligand via steric hindrance. However, many unknowns remain in this respect, and it would be of interest to explore the mutual functional effects between VWF and its passengers.

Novel aspects of VWF function: cell effector in the angiogenic process.

During the last two decades, more than 20 proteins have been identified that interact with VWF, several of them being involved in cellular signaling processes.³² Consequently, VWF has been linked to other (patho)physiological processes than haemostasis as well, including angiodysplasia, tumor metastasis and smooth muscle cell proliferation (**Figure 2**; for recent reviews on these topics see references^{32,134,135}). However, the mechanism by which

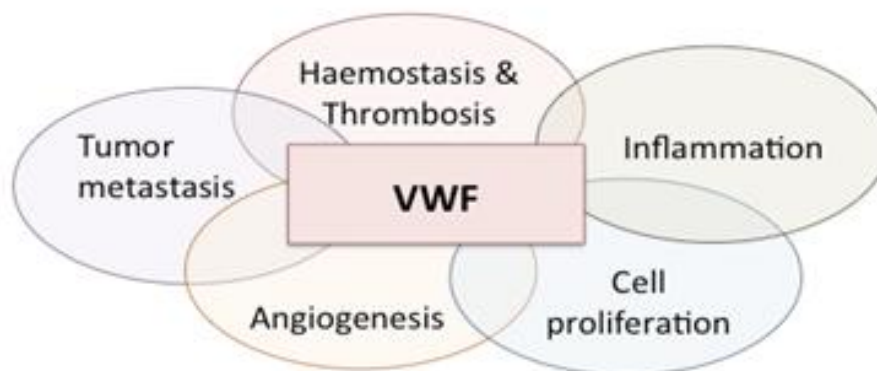


Figure 2. The functional diversity of VWF. VWF is best known for its link with the hemostatic system, where it participates in the recruitment of platelets to the injured vessel wall and acts as a carrier protein for FVIII. The physiological relevance of this function is underscored by VWF being associated with bleeding problems (VWD and acquired VW syndrome) as well as thrombotic complications (myocard infarction, stroke and venous thrombosis). More recently it has been found that VWF is involved in other patho-physiological processes as well, such as tumor metastasis (inducing tumor cell death), angiogenesis (which could provide a rationale for the relatively frequent occurrence of angiodysplasia in VWD patients), cell proliferation (associated with enhanced intima thickening after angioplasty and in CADASIL), and inflammatory processes (as observed in animal models for atherosclerosis, stroke, wound healing and experimental allergic encephalomyelitis).

VWF is linked to these processes is largely undefined. For each of the three conditions a brief overview of our current knowledge will be provided.

With regard to the angiogenic process, it has been found that the absence of VWF increases endothelial cell proliferation *in vitro*.¹³⁶ In line with this observation, VWF-deficient mice display an increased vessel density of the vasculature in the ears in comparison to VWF-expressing mice,¹³⁶ suggesting that VWF acts as a negative modulator of angiogenesis. The molecular basis of this modulatory effect is yet unclear. Results from the study by Starke and colleagues point to an effect of VWF on vascular endothelial growth factor (VEGF)-dependent angiogenesis, which proceeds via multiple intracellular and extracellular pathways dependent on $\alpha V\beta 3$ and angiopoietin-2.¹³⁶ Given that both proteins are ligands for VWF, it seems possible that VWF acts on the angiogenic process via interactions with both proteins. However, the endothelial cells contain several other VWF-binding proteins with pro- and anti-angiogenic properties, such as galectins-1 and -3,^{137,138} connective tissue growth factor¹³⁹ and insulin-like growth factor binding protein-7.¹⁴⁰ This points to a complex role of VWF, able to affect the angiogenic process at different levels.

Irrespective of the precise mechanism, the link between VWF and angiogenesis seems to be of physiological relevance, given the relatively frequent occurrence of angiodysplasia in patients with VWD.^{134,141} Angiodysplasia is characterized by vascular malformations resulting from an impaired

angiogenic process, and is often clinically manifested via gastro-intestinal bleedings.¹⁴² Interestingly, the manifestation of angiodysplasia in VWD patients is observed more frequently in patients that lack high multimers, either because of hereditary defects^{141,143} or because of acquired conditions, such as Heyde's syndrome or patients carrying circulatory assist devices.^{144,145} Why there is this specific link with high molecular weight multimers is unclear. Perhaps it involves a mechanism that is similar to the interaction between VWF and GpIb α , which also is more efficient for the larger multimers compared to smaller variants. The possibility exists that VWF interacts in a multimer size-dependent manner with so far unidentified cellular receptors (expressed on endothelial cells or other cells in the vascular wall) that are involved in maintaining the vascular integrity. Solving this enigma would be of interest for the development of novel therapeutic means to manage this severe complication of VWD.

Novel aspects of VWF function: cell effector in smooth muscle cell proliferation. Care should be taken in extrapolating the anti-proliferative effect of VWF towards VEGF-stimulated endothelial cells also to other cell types. As will be discussed in this section, VWF may also exert a proliferative effect, demonstrating that the cell effector function of VWF may be very much dependent on the local cellular environment. Upon damage of the vascular endothelial layer, VWF is able to penetrate into the intima of large peripheral vessels, where it is exposed to smooth muscle cells.¹⁴⁶⁻¹⁴⁸ The deposition of VWF in the

intima coincides with intimal thickening,¹⁴⁹ suggesting that VWF plays a role in the pathogenesis of intimal hyperplasia by promoting smooth muscle cell proliferation. This possibility is supported by *in vitro* experiments showing that VWF directly stimulates smooth muscle cell proliferation.¹⁴⁹ The transcriptional changes in smooth muscle cells that are being induced upon exposure to VWF have recently been unraveled, and involve multiple genes associated with growth factor stimulation.¹⁵⁰

The effect of VWF-dependent smooth muscle cell proliferation is not only of relevance with regard to vascular damage, for instance as a consequence of an angioplasty procedure,^{146,147} but may also be of importance in view of the hereditary disorder CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy).¹⁵⁰ The clinical phenotype of this disorder includes recurrent strokes and dementia. Analysis of brain sections of CADASIL-patients revealed that VWF is abundantly present in the brain vessels, particularly in the subarachnoid arteries that are characterized by concentric thickening of the media and adventitia.¹⁵⁰ The identification of VWF as a player in CADASIL-related smooth muscle cell proliferation could provide the basis for a novel therapeutic approach in the treatment of these patients.

Novel aspects of VWF function: cell effector in apoptosis. The versatility of VWF is nicely illustrated by the notion that VWF is not only capable of stimulating cell proliferation but also by its capacity to induce cell death. Again, it is important to take into account the local cellular environment in this regard, since the apoptotic function of VWF is probably restricted to but a few cell types. First, it was shown that VWF is able to induce platelet apoptosis via interactions with GpIb α , thereby initiating the caspase-3, Bak and Bax-dependent apoptosis pathway.¹⁵¹ The physiological consequences of this finding remain to be determined, but they could be of relevance for those conditions where there are enhanced VWF-platelet interactions without the need for thrombus formation. One such a condition could be VWD-type 2B, where gain-of-function mutations in the VWF A1 domain result in spontaneous VWF-platelet interactions.

Tumor cells are another cell type that might be susceptible to VWF-induced apoptosis. Unexpectedly, tumor cells were found to have a higher metastatic potential in VWF-deficient mice than in VWF-expressing control mice.¹⁵² This higher metastatic potential appeared to be the result of a longer survival of living cells in the absence of VWF.¹⁵² *In vitro* studies confirmed that VWF induced death of tumor cells.^{152,153} The underlying mechanism of VWF-

induced cell death remains unclear, although the observation that VWF-tumor cell interactions were mediated by α V β 3 suggest that VWF induces cell death via this integrin.¹⁵² The capacity of VWF to induce tumor cell death in an α V β 3-dependent fashion was recently confirmed in an elegant study by Mochizuki and colleagues.¹⁵⁴ However, they also identified a series of tumor cells that was capable of escaping VWF-induced cell death. The explanation for this resistance against VWF-induced apoptosis was rather unexpected: they found that tumor cells are able to secrete a protease (ADAM-28) that is able to degrade VWF.¹⁵⁴ Thus, VWF negatively regulates tumor cell survival, and certain tumor cells have armed themselves against VWF via the production of a protease that destroys the pro-apoptotic function of VWF.

Novel aspects of VWF function: a pro-inflammatory agent. The adhesive nature of the VWF protein allows it to function as a landing platform for platelets. This raises the question whether this adhesive capacity is selective for platelets, or whether also other cells are able to adhere VWF. We have previously addressed this issue, and observed that leukocytes may adhere to immobilized VWF under conditions of low shear.¹⁵⁵ In the same study, we were able to identify PSGL-1 and β 2-integrins as potential counter-receptors for VWF at the leukocyte surface.¹⁵⁵ More recently, we also identified Siglec-5 as a leukocyte receptor that is able to interact with VWF, although we did not test whether Siglec-5 contributes to leukocyte-VWF interactions under conditions of flow.¹⁵⁶ Evidence is also accumulating from other studies that VWF may actively participate in leukocyte recruitment. First, platelet-decorated VWF strings at the cellular surface efficiently attract leukocytes, even under conditions of high shear stress.¹⁵⁷ Furthermore, VWF-platelet complexes play a crucial role in the extravasation of leukocytes upon an inflammatory response.¹⁵⁸

The participation of VWF in the inflammatory response has been confirmed in several animal models for inflammation, such as atherosclerosis, wound healing, experimental allergic encephalomyelitis, and stroke.¹⁵⁹⁻¹⁶² Whether VWF plays a similar important role in the human pathology of these diseases is unclear, which could be related to the multi-factorial nature of such inflammatory conditions. For instance, VWF-deficient mice and pigs develop fewer atherosclerotic lesions compared to VWF-expressing animals, suggesting that VWF could participate in attracting leukocytes to lesion sites.^{161,163} However, human studies revealed conflicting information whether or not atherosclerosis is reduced in patients with VWD (recently reviewed by van Galen *et al.*¹⁶⁴).

One crucial difference that could explain the observed differences between humans and animals that lack VWF is that patients receive replacement therapy to replenish the reservoir of circulating VWF. As such they are less deficient in VWF compared to the animals.

Regulation of classic and new VWF functions: multimer size and shear stress. In the circulation, VWF is exposed to many of its ligands, including platelets. Therefore, mechanisms need to be in place to prevent premature interactions between VWF and platelets in order to prevent undesired vessel occlusion.¹⁶⁵ On the other hand, for some ligands (such as FVIII) it is actually necessary that VWF is able to interact with them in a constitutive manner, without a strict regulation. From these two examples it becomes clear that the versatility of VWF is not only restricted to its functions, but also with regard to the regulation of these functions.

There are two dominant mechanisms in place that contribute to the regulation of VWF function. First, VWF is able to change conformation in response to shear stress.¹⁶⁶⁻¹⁶⁸ In the normal circulation VWF is present as a globular protein, whereas exposure to increased shear forces drives the protein into an elongated conformation.¹⁶⁶⁻¹⁶⁸ This change in conformation has a number of consequences:¹⁶⁹ (1) it results in decryption of the GpIb α binding site, allowing platelet binding;¹⁰⁸ (2) the cleavage site for ADAMTS13 becomes available;^{170,171} (3) it exposes methionine residues that are sensitive to oxidation;¹⁷² (4) it promotes disulfide bridge formation between cysteine-residues in the CK-domain;^{173,174} (5) it enhances VWF self-association;¹⁷⁵ and (6) it turns VWF into a ligand for its clearance receptor LRP1.¹⁷⁶ In contrast to these shear stress-dependent phenomenon, the interactions between VWF and collagen or FVIII do not seem to require shear stress-induced conformations, as they already occur under static conditions.

Do these shear stress-induced conformational changes also affect the novel functions of VWF? In most cases, this does not seem to be the case. The effects of VWF on angiogenesis, smooth muscle cell proliferation and tumor cell death have usually been investigated *in vitro* under static conditions. Of course, this does not necessarily mean that shear stress will not affect these functions. However, additional studies are needed to get insight into the role of shear stress on novel VWF functions.

A second mechanism to regulate VWF function is to vary its multimer size, and several mechanisms are at hand to do so. One protein that contributes to the regulation of VWF multimer size is thrombospondin,

which controls VWF multimer size via the introduction of new thiols.¹⁷⁷ Second, shear stress-induced self-association may contribute to enlarge the multimer size of VWF.¹⁷⁵ However, the most dominant regulator seems to be ADAMTS13, which is able to proteolytically degrade VWF via cleavages in the A2 domain between residues Tyr1605 and Met1606.¹⁷⁸ The mechanism by which ADAMTS13 recognizes and cleaves its substrate has been described in detail in an excellent review by Crawley and colleagues.¹⁷⁹ The importance of ADAMTS13 in the regulation of VWF multimer size in view of its hemostatic properties is evident from the thrombotic complications that occur in the absence of ADAMTS13, a disorder known as thrombotic thrombocytopenic purpura.¹⁸⁰⁻¹⁸² However, does ADAMTS13 also affect non-hemostatic functions of VWF? There are indications that this is indeed conceivable. First, we already mentioned that angiodysplasia is particularly associated with VWD patients that lack high molecular weight multimers, such as in VWD-type 2A.¹⁴³ Apparently, an increased degradation of VWF interferes with the property of VWF to maintain the integrity of the vasculature. Second, increased leukocyte rolling on unstimulated veins and increased leukocyte adhesion in inflamed veins has been observed in mice deficient for ADAMTS13. Moreover, it has been found that the absence of ADAMTS13 exacerbates the inflammatory response in animal models for stroke and atherosclerosis.^{159,183-185} Apparently, proteolytic degradation of VWF by ADAMTS13 downregulates the inflammatory potential of VWF. With regard to the effect of VWF on tumor cell death, the importance of multimer size is yet unclear. It should be noted that ADAM-28 reduced VWF multimer size via proteolysis at two distinct sites in the VWF protein, which coincides with a loss in apoptotic potential.¹⁵⁴ Since these sites are located away from the α V β 3-recognition sequence (*i.e.* the RGD-motif), it seems conceivable that VWF multimer size plays a role in the interaction with tumor cells to initiate the apoptotic process.

Conclusion. Forty years after its first purification from plasma, VWF still carries many mysteries. Its versatility is steadily being exposed but even its role in thrombosis, once thought to be well understood, is still eluding us. Indeed, the notion that a VWF-mutant unable to bind α IIB β 3 is protective against thrombosis in a ferric chloride-induced model for arterial thrombosis while it is without effect in a stroke model, is a perfect example of this constant reassessment that is forced upon us.^{100,186} The possibility to target VWF in the management of thrombotic disorders should therefore be considered as a real option. With regard to the non-hemostasis functions of VWF, many avenues

also remain to be explored. The combination of data originating from both clinical and basic studies on VWF will no doubt be instrumental in expanding our knowledge of this intriguing protein.

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