von Willebrand Factor Directly Interacts With DNA From Neutrophil Extracellular Traps

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Objective—Inflammatory conditions provoke essential processes in the human vascular system. It leads to the formation of ultralarge von Willebrand factor (VWF) fibers, which are immobilized on the endothelial cell surface and transform to highly adhesive strings under shear conditions. Furthermore, leukocytes release a meshwork of DNA (neutrophil extracellular traps) during the process of the recently discovered cell death program NETosis. In the present study, we characterized the interaction between VWF and DNA and possible binding sites to underline the role of VWF in thrombosis and inflammation besides its function in platelet adhesion.

Approach and Results—Both functionalized surfaces and intact cell layers of human umbilical vein endothelial cells were perfused with isolated, protein-free DNA or leukocytes from whole blood at distinct shear rates. DNA–VWF interaction was monitored using fluorescence microscopy, ELISA-based assays, molecular dynamics simulations, and electrostatic potential calculations. Isolated DNA, as well as DNA released by stimulated leukocytes, was able to bind to shear-activated, but not inactivated, VWF. However, DNA–VWF binding does not alter VWF degradation by a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13. Moreover, DNA–VWF interaction can be blocked using unfractionated and low-molecular-weight heparin, and DNA–VWF complexes attenuate platelet binding to VWF. These findings were supported using molecular dynamics simulations and electrostatic calculations of the A1- and A2-domains.

Conclusions—Our findings suggest that VWF directly binds and immobilizes extracellular DNA released from leukocytes. Therefore, we hypothesize that VWF might act as a linker for leukocyte adhesion to endothelial cells, supporting leukocyte extravasation and inflammation. **(*Arterioscler Thromb Vasc Biol*. 2014;34:1382-1389.)**

Key Words: endothelium ■ heparin ■ inflammation ■ leukocytes ■ von Willebrand factor

Inflammation causes a release of von Willebrand factor (VWF), an adhesive glycoprotein stored in endothelial cells and platelets. It was shown that luminal-secreted VWF from Weibel–Palade bodies[1] forms ultralarge fibers, which are immobilized on the endothelial cell surface.[2] In this context, shear flow mediates VWF uncoiling, which transforms VWF to a highly adhesive protein by exposing the A1- and A2-domains, which features specific binding sites for heparin, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13), and glycoprotein 1bα (GP1bα).[3–5] The latter is involved in VWF–platelet interaction. Platelet adhesion to VWF has been shown to play an essential role in primary hemostasis and the formation of deep vein thrombosis in mouse models.[6]

Also, heparin binds to parts of the A1-domain.[4,7] Besides naturally occurring unfractionated heparin, there are different kinds of low-molecular-weight heparins on the market, which differ in molecular properties and in their mode of action. The length of these heparins varies significantly. For example, unfractionated heparin has a molecular weight of 15 kDa, whereas the smallest low-molecular-weight heparin, Fondaparinux, contains only 5 sugar residues. However, all heparins are highly negatively charged.

Besides the aforementioned VWF release, inflammatory conditions provoke the generation of extracellular DNA traps, which have recently been discovered as large DNA fibers generated by leukocytes, especially neutrophils (neutrophil extracellular traps, NETs).[8] The recently described mechanism, called NETosis, which is distinct from apoptosis or necrosis,[9] implicates that leukocytes are able to release a meshwork of chromosomal DNA, which includes histones and granular antimicrobial

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Nonstandard Abbreviations and Acronyms	
ADAMTS13	a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
NET	neutrophil extracellular trap
VWF	von Willebrand factor

proteins, such as myeloperoxidase or neutrophil elastase, to trap and kill microorganisms.[8,9] Extracellular DNA traps have been linked to several diseases, including sepsis,[10] small-vessel vasculitis,[11] or preeclampsia.[12] Other studies showed that both extracellular DNA traps and VWF are significantly involved in deep vein thrombosis.[6,13–15] It has been demonstrated that there is a correlation between levels of circulating nucleosomes, activated neutrophils, and the occurence of deep vein thrombosis.[16] Also, immunostained sections of venous thrombi from animal models indicate a connection of inflammation and thrombosis by the fact that both NETs and VWF seem in close proximity to each other.[13,14] This association of both molecules was linked to the capability of VWF to bind histones.[17]

Here we show that VWF and pure, isolated DNA from human whole blood are able to interact in a shear-dependent manner, but independently of histones or other proteins. We examined the interaction of ultralarge VWF and extracellular DNA traps under flow conditions and analyzed the characteristics of the binding process by applying an immunobased binding assay and immunostaining. We hypothesize that VWF, by binding DNA, might act as a linker for leukocyte adhesion to the endothelium and thereby underline the role of VWF in inflammation, besides its function in platelet adhesion.

Materials and Methods

Materials and Methods are available in the online-only Supplement.

Results

DNA Binds to VWF Under Shear Conditions

To investigate whether VWF can bind to isolated DNA molecules, we studied the binding of DNA to physiologically released ultralarge VWF fibers on a histamine-stimulated, confluent endothelial cell layer under shear flow conditions of 2 to 20 dyne/cm^2. Additionally, we applied a microfluidic channel system using VWF functionalized surfaces, which were perfused with pure, isolated DNA from whole blood. Protein contamination of isolated DNA was excluded by

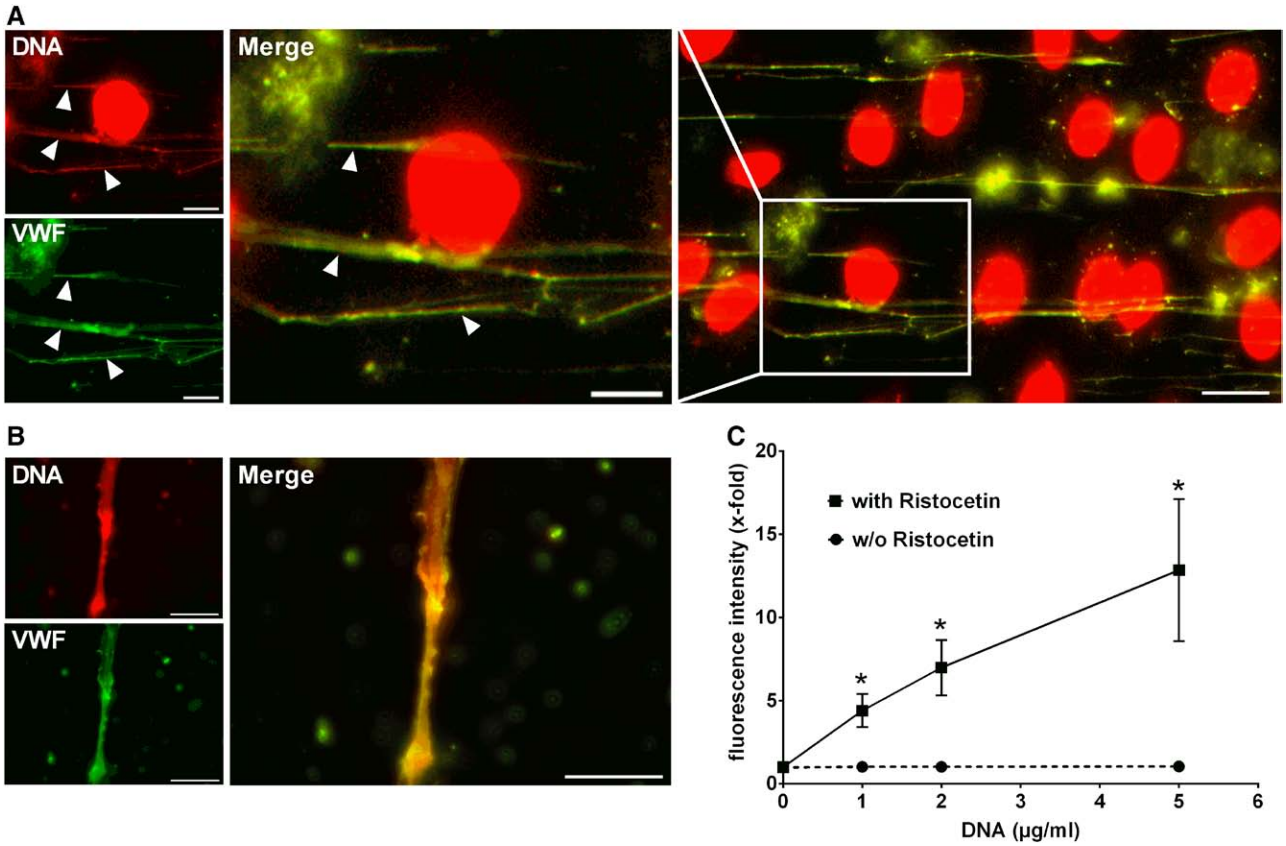

Figure 1. DNA binds to von Willebrand factor (VWF) under shear conditions. **A**, Protein-free DNA was perfused at 10 dyne/cm^2 over a histamine-stimulated, intact endothelial cell layer (thereby releasing VWF fibers) and labeled with Sytox Green (visualized in red). DNA colocalized with released VWF strings (arrowheads). Also, nuclei of human umbilical vein endothelial cells were stained during the fixation process because of excessive dye in the supernatant. Green indicates VWF; and red, DNA. Scale bar, 10 µm. **B**, Isolated DNA was perfused over a functionalized VWF surface at 2 dyne/cm^2. 4′,6-Diamidino-2-phenylindole–labeled DNA (visualized in red) binds to VWF fibers only (green), but not globular, nonactivated VWF, under flow conditions. Scale bar, 50 µm. **C**, Binding of DNA to VWF is Ristocetin-dependent; increasing DNA concentrations (0–5 µg/mL) were incubated with 1 µg/mL VWF in the presence (solid line) or absence (dotted line) of 1.5 mg/mL Ristocetin. The binding of DNA to VWF was assessed by fluorescence intensity measurments using Sytox Green labeling. Data are expressed as mean±SD (*P<0.05; n=6).

absorbance measurements of 260/280 nm ratio, Bradford assay, and gel electrophoresis. In both cases, immunofluorescence staining showed a clear colocalization of perfused DNA with either cellular-released VWF fibers or surface-coated VWF (Figure 1A and 1B; Movie I in the online-only Data Supplement). DNA did not interact with BSA-coated surfaces or globular, nonactivated VWF (data not shown). No difference in binding could be observed at different levels of shear stress between 2 and 20 dyne/cm^2, so we assumed that already 2 dyne/cm^2 is sufficient to promote DNA–VWF interaction (data not shown). These results indicate that DNA is able to bind directly to VWF under shear conditions without the involvement of other proteins.

To characterize the binding of DNA to VWF in more detail, we performed an immunobased quantitative assay with and without Ristocetin, an antibiotic thought to expose the binding sites of the A1-domain of VWF in the absence of high shear forces.[18] Both DNA and VWF molecules were incubated in increasing DNA/VWF ratios in the absence or presence of Ristocetin at different concentrations, and DNA binding was examined. DNA binding to VWF was only detectable when both molecules were coincubated with 1.5 mg/mL Ristocetin (Figure 1C) and decreased with descending Ristocetin concentrations (Figure I in the online-only Data Supplement). These data are in line with our abovementioned data and demonstrate that DNA binding to VWF is dependent on an exposed VWF A1-domain and occurs only on activated, but not on globular, VWF.

GP1bα-Binding Site of VWF A1-Domain Is Blocked by DNA

As mentioned above, isolated DNA directly binds to VWF in a shear- or Ristocetin-dependent manner. Therefore, we next analyzed the binding capacity of DNA to recombinant VWF A1-domain and the effect of DNA–VWF interaction on other binding partners of VWF. As shown in Figure 2A, DNA is able to bind to an isolated A1-domain in the presence of Ristocetin. Previous studies proved that the binding of large polyanionic molecules, such as heparin, to VWF leads to a decreased adhesion of platelets.[19] Therefore, we studied the effect of the polyanionic DNA on platelet–VWF binding. Perfusion of platelet-rich plasma over a DNA-preincubated VWF surface demonstrated that the binding of platelets to VWF was significantly impaired by 78% (±6.8%) compared with control experiments lacking DNA (Figure 2B and 2C). Furthermore, this DNA blockage could not only be observed on a wild-type VWF coating but also on an A1-domain functionalized channel (see Figure 2B). The subsequent reduction in platelet binding is comparable with the amount of adhesion on a VWF-mutant protein that lacks the A1-domain (Figure 2B). However, the rolling velocity of residual platelets on the DNA-bound wild-type VWF surface remained unchanged (data not shown). Although these in vitro flow experiments are somewhat artificial, this result indicates a hindrance in VWF–platelet interaction because of a blockage of the GP1bα-binding site in the VWF A1-domain by DNA. However, it should be mentioned that under physiological conditions, in which other blood components such as erythrocytes are present, this blockage can be restored in a hematocrit-dependent manner because of the rheological distribution of the platelets (Figure IIA and IIB in the online-only Data Supplement). This phenomenon has also been described in a previous work, where it was shown that the adhesion probability of platelets to VWF is strongly dependent on hematocrit.[20]

DNA Binding to VWF Is Blocked by Heparin

Because of our previous results, we hypothesized that the VWF A1-domain is mainly involved in the binding process of DNA to VWF. To delineate the contribution of heparin-binding sites in the A1-domain, we assessed whether DNA binding to VWF can be blocked using unfractionated heparin. Therefore, plasmatic VWF was immobilized on a microfluidic

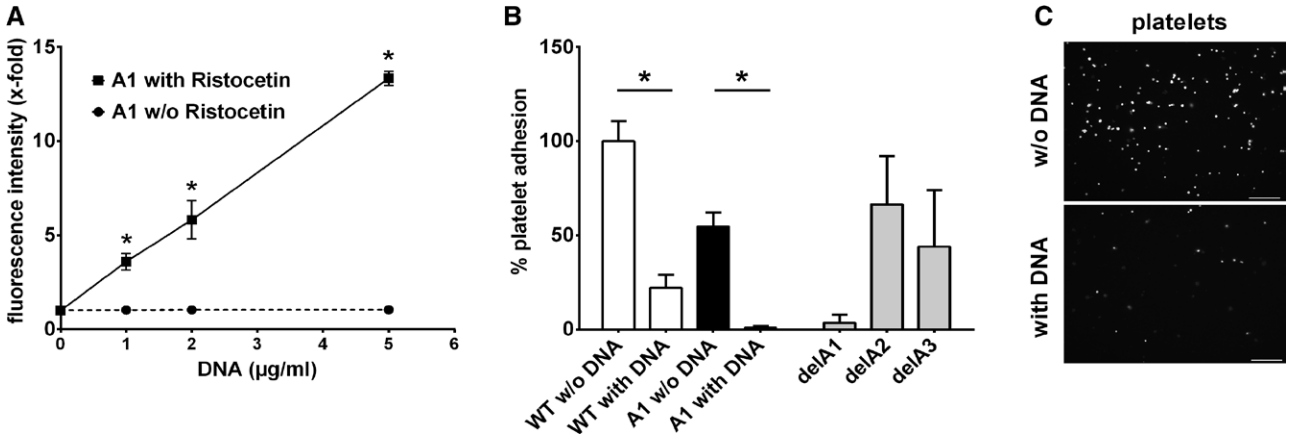

Figure 2. Glycoprotein 1bα-binding site is blocked by DNA. **A**, DNA directly binds to the recombinant von Willebrand factor (VWF) A1-domain. Increasing DNA concentrations (0–5 μg/mL) were incubated with 1 μg/mL VWF A1-domain in the presence (solid line) or absence (dotted line) of 1.5 mg/mL Ristocetin. The binding of DNA to VWF A1-domain was assessed by fluorescence intensity measurments using Sytox Green labeling. Data are expressed as mean±SD (*P<0.05; n=3). **B**, Quantification of platelets adhering to a VWF wild-type (WT) surface that was blocked with DNA shows a significant reduction by 78% (±6.8%) in platelets binding to VWF. In addition, platelet binding to the A1-domain was completely blocked by DNA. delA1, delA2, and delA3 indicate VWF proteins that lack the A1-, A2-, and A3-domains, respectively. Data are expressed as mean±SD (*P<0.05; n=3–6). **C**, Platelet-rich plasma was perfused over a VWF surface that was blocked with 10 μg/mL isolated DNA. Adhering platelets were stained using an anti-GP1bα antibody. White indicates platelets. Scale bar, 50 μm.

channel and was preincubated with 50 U/mL unfractionated heparin before perfusion with isolated DNA. Indeed, immunofluorescence staining confirmed that the binding of DNA to VWF was completely abolished compared with a control channel (Figure 3A and 3B). Because the DNA was proven to be protein-free, the involvement of histones could be excluded. DNA that was once immobilized on a VWF surface could be removed with DNAse I, but not with heparin (Figure IIIA and IIIB in the online-only Data Supplement), indicating that the binding occurs with a high affinity. This result could also be accomplished in an ELISA-based binding assay (Figure IIIC in the online-only Data Supplement).

In addition, we analyzed whether autogenic DNA of *Staphylococcus aureus*– or phorbol 12-myristate 13-acetate (PMA)–stimulated neutrophils from whole blood can attach to a VWF functionalized surface under flow conditions, and whether this interaction can be blocked by heparin. For this experiment, neutrophils were stimulated to release NETs and afterward perfused over a microfluidic channel. In the control channel, both *S. aureus*– (data not shown) and PMA-treated neutrophils released a massive network of DNA fibers, which were captured by the VWF surface. Thereby, the DNA meshwork and several neutrophilic cells were immobilized on the surface, and secondary neutrophils were trapped (Figure 3C). Perfusion of 100 U/mL DNAse I over the formed DNA network completely dismanteled all DNA fibers and removed all attaching neutrophils (Movie II in the online-only Data Supplement). However, if the VWF-coated surface was blocked with 50 U/mL heparin beforehand, neutrophils that released their DNA could not attach to the surface, and leukocyte adhesion to VWF was almost completely impaired (Figure 3D). Because, for this experiment, it was an autogenic release of NETs from neutrophils, an involvement of histones cannot be excluded in this case. Nevertheless, this shows that heparin is able to block the DNA and leukocyte binding to VWF, indicating the same binding site of heparin and DNA within the A1-domain.

Inhibition Efficiency of DNA–VWF Binding Varies for Different Heparins

Using the abovementioned immunobased assay together with Ristocetin, we applied different commercially available heparins to block DNA binding to VWF in vitro. Unfractionated heparin almost completely inhibited DNA–VWF interaction, whereas low-molecular-weight heparins such as Tinzaparin (6.5 kDa on average) or Fondaparinux (a pentasaccharide)

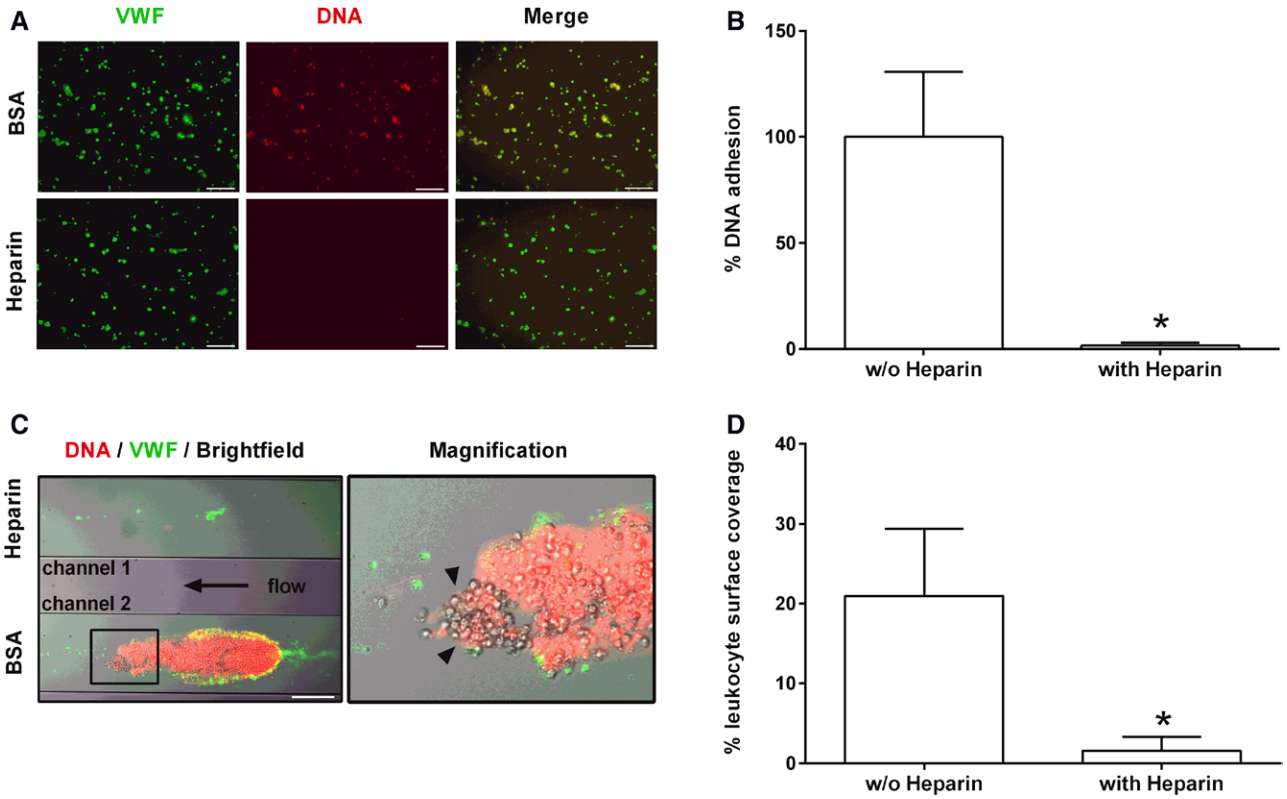

Figure 3. Binding of DNA to von Willebrand factor (VWF) is blocked by heparin. **A**, Isolated DNA was perfused over a VWF surface. Bound DNA was visualized with 4′,6-diamidino-2-phenylindole (visualized in red). Immunofluorescence staining shows a decrease in DNA binding after blockage of the surface with 50 U/mL heparin compared with a 2% BSA–blocked control surface. Green indicates VWF; and red, DNA. Scale bar, 50 μm. **B**, Quantification of adhering DNA on a VWF channel that was blocked with heparin. Data are expressed as mean±SD (*P<0.05; n=5). **C**, Isolated and phorbol 12-myristate 13-acetate–stimulated leukocytes were perfused through 2 opposing VWF channels. Autolog-released DNA from leukocytes was not able to be immobilized on a VWF surface that has previously been blocked with 50 U/mL heparin (channel 1). Blocking with 2% BSA had no influence on DNA adhesion to VWF (channel 2). Magnification shows the indicated box in the left panel, representing adhering leukocytes to the VWF surface (arrowheads). Green indicates VWF; and red, DNA. Scale bar, 200 μm. **D**, Quantification of adhering leukocytes to a VWF channel in the absence and presence of heparin. Data are expressed as mean±SD (*P<0.05; n=3).

were less effective (Figure 4A and 4B). Preincubation of VWF with Tinzaparin significantly reduced the binding of DNA, whereas Fondaparinux, the smallest heparin available, kept DNA–VWF binding unchanged. After partial digestion of unfractionated heparin using heparinase I, the DNA–VWF interaction could be restored (Figure 4C and 4D). These results indicate that an inhibition of DNA–VWF binding depends on the length of negatively charged molecules. To further confirm the charge-dependent interaction of negative DNA with the positive A1-domain, we neutralized the negative charge of the DNA by polycationic chitosan. It is well known that chitosans are able to interact with polyanions such as DNA.[21] Indeed, chitosan-mediated blockage of negative charges on DNA inhibits its binding to VWF in a concentration-dependent manner (Figure V in the online-only Data Supplement), which strengthens our hypothesis that DNA binding to VWF is primarily charge-based.

VWF A1-Domain Is a Potential Binding Site for DNA

Experiments suggest a competitive binding between GP1bα, heparin, and DNA to VWF. Because binding sites of GP1bα

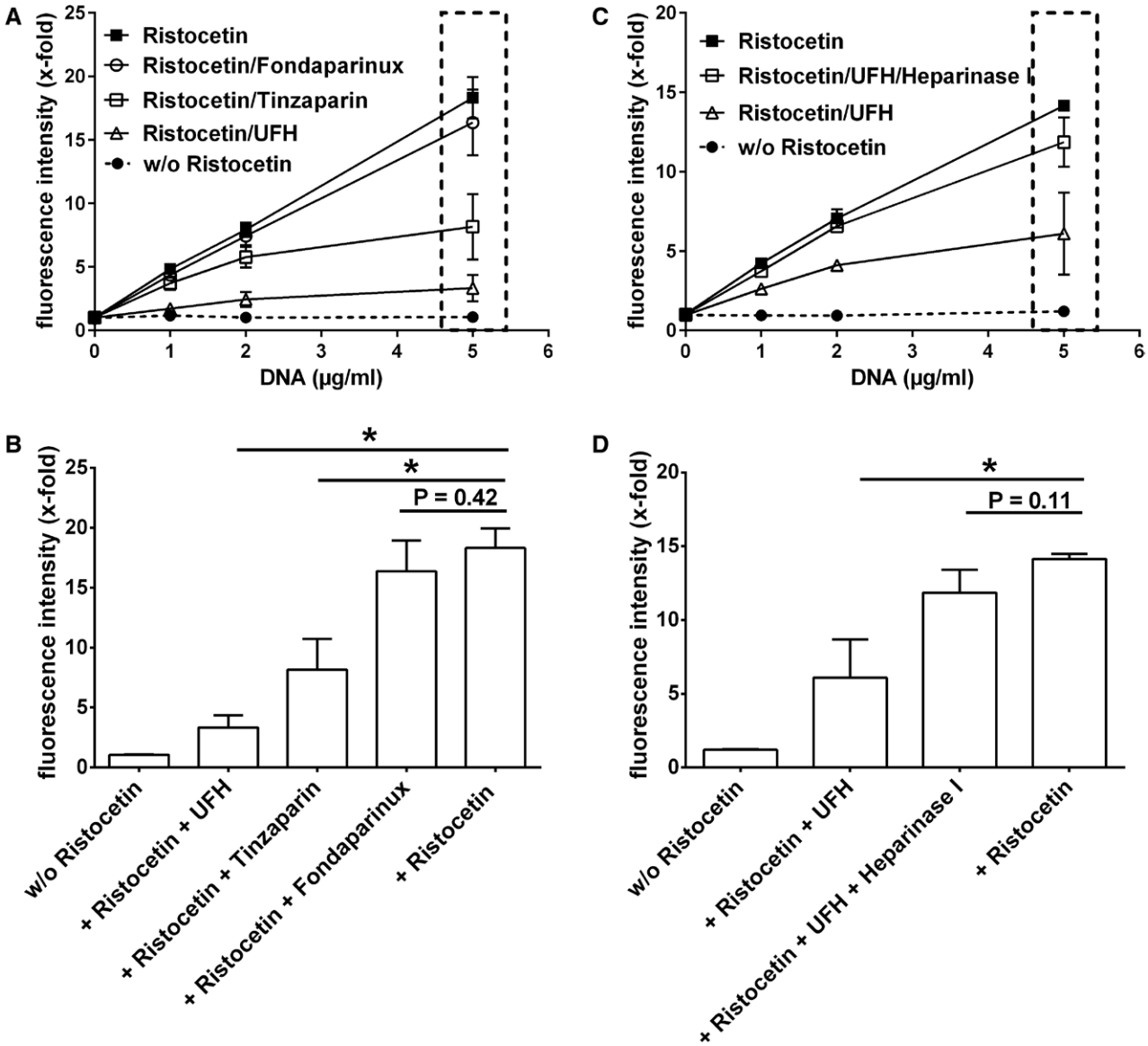

Figure 4. Binding of DNA to von Willebrand factor (VWF) is blocked by low-molecular-weight heparins. **A**, Increasing concentrations (0–5 µg/mL) of DNA were incubated with 1 µg/mL VWF, which was preincubated with 50 U/mL unfractionated heparin (UFH), 50 U/mL Tinzaparin, or 29 µg/mL Fondaparinux (both low-molecular-weight heparins). Binding of DNA to VWF was assessed by Sytox Green fluorescence intensity increase. The dotted box is represented as a bar graph in **B** to indicate significant blockage. **B**, Preincubation of VWF with UFH and Tinzaparin, but not with Fondaparinux, causes a significant decrease in DNA binding. Data are expressed as mean±SD (*P<0.05; n=3). **C**, Increasing concentrations (0–5 µg/mL) of DNA were incubated with 1 µg/mL VWF, which was preincubated with 50 U/mL UFH, or UFH that has been digested by heparinase I beforehand. Binding of DNA to VWF was assessed by Sytox Green fluorescence intensity increase. The dotted box is represented as a bar graph in **D** to indicate significant blockage. **D**, Preincubation of VWF with UFH causes a significant decrease in DNA binding, which can be restored by partial heparinase I digestion of UFH. Data are expressed as mean±SD (*P<0.05; n=3).

and heparin are located within the A1-domain, we calculated the electrostatic potential produced by this domain to search for positively charged regions where the negatively charged DNA could potentially bind. The electrostatic potential revealed a positively charged region at the A1-domain (Figure 5A), which remained approximately constant during the course of a 195-ns molecular dynamics simulation (Figure IVA in the online-only Data Supplement). This positively charged area intersects with a part of the GP1bα-binding site (compare blue in Figure 5A with orange in Figure 5B) and contains the residues associated with the heparin-binding site (compare blue in Figure 5A with green in Figure 5C). This suggests that DNA interacts with VWF at the same regions that GP1bα and heparin do.

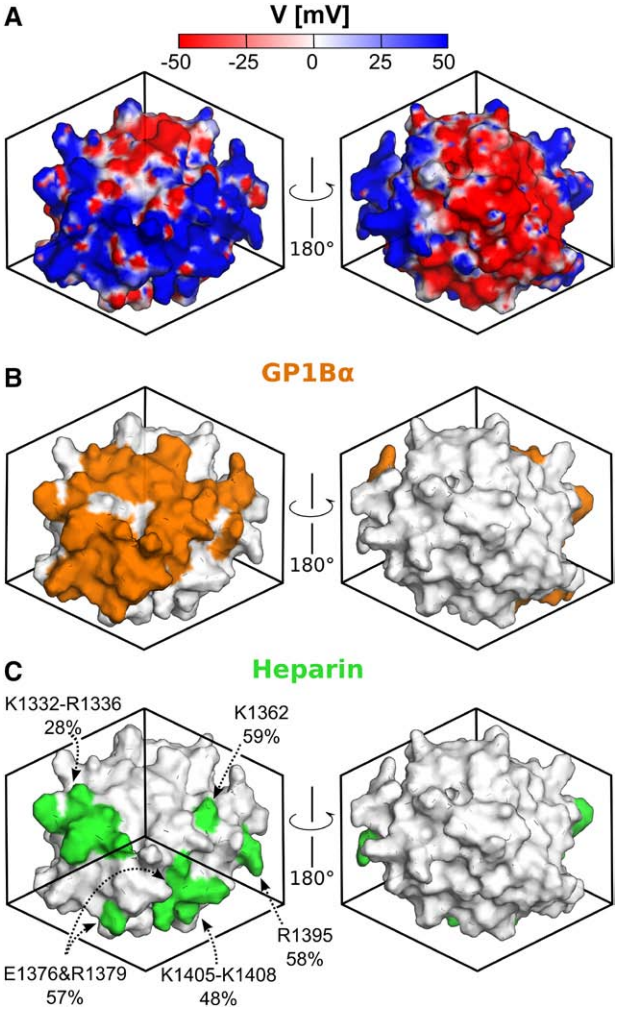

Figure 5. von Willebrand factor (VWF) A1-domain is a potential binding site for DNA **A**, Electrostatic potential generated by the VWF A1-domain. Potentials were calculated for 10 representative conformations of the VWF A1-domain extracted from a 195-ns molecular dynamics simulation. Here, 1 of the resulting potentials is shown (for all 10 calculated potentials, see Figure IV in the online-only Data Supplement). The potential is depicted at a distance of 1.4 Å from the protein, according to the color scale at the top side. **B** and **C**, Glycoprotein 1bα (GP1bα)– (**B**) and heparin- (**C**) binding regions in the VWF A1-domain. The VWF A1-domain is shown in surface representation. VWF A1 residues located at the GP1bα–VWF A1 interface are highlighted in orange in **B**. VWF A1 residues associated with heparin binding are shown in green in **C**. The reduction of heparin binding because of the replacement of these residues is indicated by the percentage values and was taken from Adachi et al.[4]

DNA Does Not Block the Cleavage Site for ADAMTS13

Because we found that DNA binds to the VWF A1-domain, we intended to ascertain whether this interaction could possibly block also parts of the A2-domain, which contains the cleavage site for ADAMTS13. Therefore, we performed in vitro flow experiments for DNA binding to VWF fibers released by a confluent human umbilical vein endothelial cell layer with or without the addition of ADAMTS13. After immunofluorescence staining, we could see that even in the presence of DNA, VWF fibers were removed by ADAMTS13 activity (Figure 6A and 6B). Although we saw the same amount of fibers in the end point observation of the microfluidic experiments, we observed a decreased cleavage capacity of ADAMTS13 in the time course of an ELISA activity (Figure 6C). In addition, electrostatic potential calculations revealed that the surface around the A2-domain is mostly negatively charged (see Figure IVB in the online-only Data Supplement), which may disfavor the interaction of this domain with DNA. Surprisingly, in the presence of DNA, more VWF fibers were detectable. Using DNAse I, the increase in VWF string formation could be significantly reduced (Figure 6A). The latter results indicate that the DNA may support the activation and stabilization of VWF fibers on shear flow conditions, similar to previous results where platelet-bound VWF has been shown to be a better ADAMTS13 substrate.[22]

Discussion

In this work, we could show that protein-free DNA is able to directly bind to VWF. Our data extend to the already existing in vivo measurements in deep vein thrombosis mouse models, that plasma DNA levels are increased and that VWF and DNA released by leukocytes (NETs) overlap in thrombus sections.[13,14] However, these previous findings were correlated to the observation that proteins such as histones are still present on the autolog-released NETs and that these proteins are then able to interact with VWF.[17] In contrast, our findings demonstrate that the colocalization of VWF with DNA is not necessarily dependent on other proteins, but instead pure isolated DNA, which is protein- and RNA-free, is able to bind directly to VWF and mediate leukocyte adhesion under physiological blood flow conditions (Figures 1A and 3C). Consequently, the DNA–VWF interaction could not only lead to thrombus formation but additionally promote leukocyte extravasation. In this context, it was recently demonstrated that blocking of VWF decreases leukocyte extravasation in different inflammatory mouse models and in immune complex–mediated vasculitis, which may open a new anti-inflammatory treatment option.[23,24] What is more, we previously showed that *S. aureus* itself binds to the VWF A1- and A3-domains, which supports *S. aureus* adhesion to the vessel wall and, in turn, can be blocked using heparin.[25] Besides this process, DNA release by leukocytes can be induced by *S. aureus* infection. Hence one may imagine a scenario where *S. aureus*–induced NET formation leads to DNA–VWF–*Staphylococcus* binding, followed by leukocyte

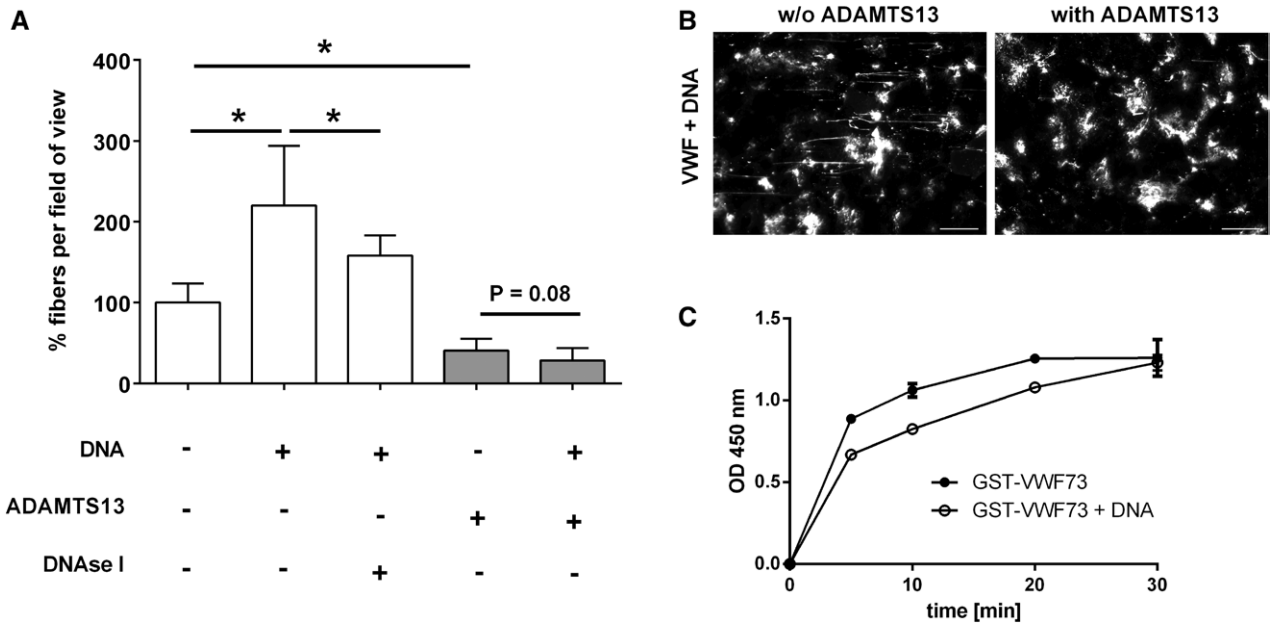

Figure 6. DNA binding does not inhibit von Willebrand factor (VWF) cleavage by a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13). **A**, DNA significantly increases the amount of VWF strings in the absence of ADAMTS13, which can be prevented by DNAse I. However, both in the presence and absence of DNA on VWF fibers, the number of VWF fibers per field of view is significantly decreased by ADAMTS13. There is no significant difference in the amount of strings after the addition of ADAMTS13 in both cases. Data are expressed as mean±SD (*P<0.05; n=3). **B**, Immunofluorescence images showing that even in the presence of DNA, ADAMTS13 is able to cleave VWF fibers at 10 dyne/cm^2. Without ADAMTS13, large VWF strings are visible on human umbilical vein endothelial cells. These fibers are degraded in the presence of ADAMTS13. White indicates VWF. Scale bar, 50 μm. **C**, ADAMTS13 activity was measured using an ELISA that quantifies cleavage by glutathion-S-transferase–tagged VWF73 (GST-VWF73) products. In the presence of DNA, ADAMTS13 cleavage is slowed down (open dots) but, however, reaches the same level after 30 min as without DNA (filled dots).

adhesion to the vessel wall and consecutive extravasation. Furthermore, a deficiency in the VWF-regulating enzyme, ADAMTS13, leads to an increased leukocyte adhesion and enhanced extravasation of neutrophils.[26] The binding of leukocyte-derived extracellular DNA to VWF, therefore, represents a new possibility for leukocytes to be recruited to the vessel wall, besides the suggested direct binding to VWF via P-selectin glycoprotein ligand-1 and β2 integrins.[27]

However, our data show that this interaction strongly depends on shear flow. Uncoiling of the VWF molecule occurs under flow conditions above a critical shear rate,[28] which can expose binding sites such as the A1-domain. In the absence of flow conditions, Ristocetin is an antibiotic to mimic this state–function relationship without the application of shear flow in an in vitro setup. In both cases, we could detect a binding of DNA to VWF; however, if both prerequisites are absent, an interaction of the molecules is not possible. These findings suggest that DNA binds only to an uncoiled VWF and, therefore, to its A1-domain.

Our studies suggest that binding occurs via electrostatic interactions between the polyanionic molecule (DNA) and the positively charged VWF A1-domain, but has no effect on the VWF A2-domain, because the specific enzyme, ADAMTS13, is still able to cleave VWF fibers. It is well known that the A1-domain of VWF exhibits a main role in the function of the large VWF molecule. Many binding sites are buried at this location, especially the binding sites for both platelet GP1bα and heparin. All these partners, examined in our experiments, are negatively charged or have at least several negatively charged residues, so that they may be classified as polyanionic molecules that are able to bind to the positively charged VWF A1-domain.[7] In particular, the phosphate groups of the DNA backbone and the sulfate groups linked to the heparin sugars make up the similar polyanionic character of both biopolymers. However, low-molecular-weight heparins and heparinase-digested unfractionated heparin instead show less capability to block DNA–VWF binding, which suggests that the impaired interaction is because of molecule size and the amount of negative charges.

Heparins are known to be multifunctional glycosaminoglycans, which are preferred anticoagulants for venous thromboembolism treatment and the first choice of therapy for venous thromboembolism in patients with cancer.[29] The classic mode of action is that heparins are able to bind antithrombin III and thereby accelerate the blockage of thrombin and mainly factor Xa. However, as shown in this work, heparins are also able to directly interact with VWF, thereby blocking DNA binding to VWF, which demonstrates that the prothrombotic effect of extracellular DNA traps[6,13,14] could be diminished by heparin. In conclusion, our data add a new aspect to the anticoagulatory mechanisms of heparin, which may further explain the beneficial effect of heparin in the treatment of venous thromboembolism, expecially in patients suffering from cancer.

Our experiments propose a new general function of the VWF molecule, namely to act as a polyanion-binding molecule after uncoiling by interacting with highly negatively charged molecules such as heparin or DNA, however being nonspecific but with a high affinity. This DNA–VWF interaction may play a pivotal role in leukocyte adhesion and extravasation on inflammation and coagulatory conditions.

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Disclosures

None.

References

1. Nightingale T, Cutler D. The secretion of von Willebrand factor from endothelial cells; an increasingly complicated story. *J Thromb Haemost*. 2013;11(suppl 1):192–201.
2. Goerge T, Niemeyer A, Rogge P, Ossig R, Oberleithner H, Schneider SW. Secretion pores in human endothelial cells during acute hypoxia. *J Membr Biol*. 2002;187:203–211.
3. Dong JF, Moake JL, Nolasco L, Bernardo A, Arceneaux W, Shrimpton CN, Schade AJ, McIntire LV, Fujikawa K, López JA. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood*. 2002;100:4033–4039.
4. Adachi T, Matsushita T, Dong Z, Katsumi A, Nakayama T, Kojima T, Saito H, Sadler JE, Naoe T. Identification of amino acid residues essential for heparin binding by the A1 domain of human von Willebrand factor. *Biochem Biophys Res Commun*. 2006;339:1178–1183.
5. 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*. 1983;72:1–12.
6. Brill A, Fuchs TA, Chauhan AK, Yang JJ, De Meyer SF, Köllnberger M, Wakefield TW, Lämmle B, Massberg S, Wagner DD. von Willebrand factor-mediated platelet adhesion is critical for deep vein thrombosis in mouse models. *Blood*. 2011;117:1400–1407.
7. Rastegar-Lari G, Villoutreix BO, Ribba AS, Legendre P, Meyer D, Baruch D. Two clusters of charged residues located in the electropositive face of the von Willebrand factor A1 domain are essential for heparin binding. *Biochemistry*. 2002;41:6668–6678.
8. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. *Science*. 2004;303:1532–1535.
9. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, Weinrauch Y, Brinkmann V, Zychlinsky A. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol*. 2007;176:231–241.
10. McDonald B, Urrutia R, Yipp BG, Jenne CN, Kubes P. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe*. 2012;12:324–333.
11. Kessenbrock K, Krumbholz M, Schönermarck U, Back W, Gross WL, Werb Z, Gröne HJ, Brinkmann V, Jenne DE. Netting neutrophils in autoimmune small-vessel vasculitis. *Nat Med*. 2009;15:623–625.
12. Gupta A, Hasler P, Gebhardt S, Holzgreve W, Hahn S. Occurrence of neutrophil extracellular DNA traps (NETs) in pre-eclampsia: a link with elevated levels of cell-free DNA? *Ann N Y Acad Sci*. 2006;1075:118–122.
13. Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD Jr, Wrobleski SK, Wakefield TW, Hartwig JH, Wagner DD. Extracellular DNA traps promote thrombosis. *Proc Natl Acad Sci U S A*. 2010;107:15880–15885.
14. Brill A, Fuchs TA, Savchenko AS, Thomas GM, Martinod K, De Meyer SF, Bhandari AA, Wagner DD. Neutrophil extracellular traps promote deep vein thrombosis in mice. *J Thromb Haemost*. 2012;10:136–144.
15. Fuchs TA, Brill A, Wagner DD. Neutrophil extracellular trap (NET) impact on deep vein thrombosis. *Arterioscler Thromb Vasc Biol*. 2012;32:1777–1783.
16. van Montfoort ML, Stephan F, Lauw MN, Hutten BA, Van Mierlo GJ, Solati S, Middeldorp S, Meijers JC, Zeerleder S. Circulating nucleosomes and neutrophil activation as risk factors for deep vein thrombosis. *Arterioscler Thromb Vasc Biol*. 2013;33:147–151.
17. Ward CM, Tetaz TJ, Andrews RK, Berndt MC. Binding of the von Willebrand factor A1 domain to histone. *Thromb Res*. 1997;86:469–477.
18. Hoylaerts MF, Nuyts K, Peerlinck K, Deckmyn H, Vermylen J. Promotion of binding of von Willebrand factor to platelet glycoprotein Ib by dimers of ristocetin. *Biochem J*. 1995;306(Pt 2):453–463.
19. Sobel M, McNeill PM, Carlson PL, Kermode JC, Adelman B, Conroy R, Marques D. Heparin inhibition of von Willebrand factor-dependent platelet function *in vitro* and in vivo. *J Clin Invest*. 1991;87:1787–1793.
20. Chen H, Angerer JI, Napoleone M, Reininger AJ, Schneider SW, Wixforth A, Schneider MF, Alexander-Katz A. Hematocrit and flow rate regulate the adhesion of platelets to von Willebrand factor. *Biomicrofluidics*. 2013;7:64113.
21. Maurstad G, Danielsen S, Stokke BT. The influence of charge density of chitosan in the compaction of the polyanions DNA and xanthan. *Biomacromolecules*. 2007;8:1124–1130.
22. Chen J, Ling M, Fu X, López JA, Chung DW. Simultaneous exposure of sites in von Willebrand factor for glycoprotein Ib binding and ADAMTS13 cleavage: studies with ristocetin. *Arterioscler Thromb Vasc Biol*. 2012;32:2625–2630.
23. Petri B, Broermann A, Li H, Khandoga AG, Zarbock A, Krombach F, Goerge T, Schneider SW, Jones C, Nieswandt B, Wild MK, Vestweber D. von Willebrand factor promotes leukocyte extravasation. *Blood*. 2010;116:4712–4719.
24. Hillgruber C, Steingräber AK, Pöppelmann B, Denis CV, Ware J, Vestweber D, Nieswandt B, Schneider SW, Goerge T. Blocking von Willebrand factor for treatment of cutaneous inflammation. *J Invest Dermatol*. 2014;134:77–86.
25. Pappelbaum KI, Gorzelanny C, Grässle S, Suckau J, Laschke MW, Bischoff M, Bauer C, Schorpp-Kistner M, Weidenmaier C, Schneppenheim R, Obser T, Sinha B, Schneider SW. Ultralarge von Willebrand factor fibers mediate luminal Staphylococcus aureus adhesion to an intact endothelial cell layer under shear stress. *Circulation*. 2013;128:50–59.
26. Chauhan AK, Kisucka J, Brill A, Walsh MT, Scheiflinger F, Wagner DD. ADAMTS13: a new link between thrombosis and inflammation. *J Exp Med*. 2008;205:2065–2074.
27. Pendu R, Terraube V, Christophe OD, Gahmberg CG, de Groot PG, Lenting PJ, Denis CV. P-selectin glycoprotein ligand 1 and beta2-integrins cooperate in the adhesion of leukocytes to von Willebrand factor. *Blood*. 2006;108:3746–3752.
28. Schneider SW, Nuschele S, Wixforth A, Gorzelanny C, Alexander-Katz A, Netz RR, Schneider MF. Shear-induced unfolding triggers adhesion of von Willebrand factor fibers. *Proc Natl Acad Sci U S A*. 2007;104:7899–7903.
29. Brose KM, Lee AY. Cancer-associated thrombosis: prevention and treatment. *Curr Oncol*. 2008;15(suppl 1):S58–S67.

Significance

It was recently shown that von Willebrand factor–DNA interaction plays an important role in inflammation and deep vein thrombosis. Using advanced microfluidics, molecular biological tools, and electrostatic potential calculations, the present work unravels the molecular mechanism of this interaction. We could show, for the first time to our knowledge, that isolated, pure (protein-free) DNA binds to the A1-domain of von Willebrand factor, which can be blocked by heparins. Hence, heparin treatment might be of superior benefit for patients with thrombosis known to exhibit high amounts of DNA and von Willebrand factor, as also reflected in recent therapeutic guidelines for cancer-related thrombosis. Therefore, our study emphasizes the effect of von Willebrand factor's binding capacity to highly negatively charged molecules and considerably underlines the role of von Willebrand factor in both inflammation and thrombosis.