Interaction of von Willebrand Factor with Staphylococcus aureus

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Intravascular infection due to Staphylococcus aureus requires colonization of subendothelium in the presence of shear forces. von Willebrand factor (VWF) is a large multimeric glycoprotein playing a key role in platelet adhesion to subendothelium. To determine whether VWF may also play a role in adhesion of S. aureus to endovascular sites, binding of VWF to S. aureus and adhesion of S. aureus to VWF-adsorbed substrates was examined. Binding isotherms revealed a dose-dependent reaction of purified VWF with S. aureus Cowan 1 as well as VWF binding to other S. aureus strains. On solid phase, VWF showed saturable adsorption kinetics to polymethylmethacrylate and promoted S. aureus adhesion up to 67-fold in a trypsin-sensitive reaction. Similar adhesion promotion was observed when recombinant VWF was used. These results show that VWF interacts with S. aureus in suspension and promotes S. aureus adhesion to surfaces, suggesting a role of VWF in the pathogenesis of intravascular S. aureus infections.

Endovascular metastatic complications, in particular infective endocarditis, are among the most serious complications of staphylococcal septicemia resulting from access of Staphylococcus aureus to the circulatory system, frequently after minor trauma, surgery, or insertion of intravascular devices [1]. To initiate intravascular infection, staphylococci must first adhere to host factors exposed on endovascular substrates. While significant progress has been made in the molecular characterization of S. aureus adhesins [2–8] and in understanding the genetic systems regulating their expression [9–12], the mechanisms by which S. aureus initiates colonization of endovascular sites are not completely understood. Furthermore, despite availability of potent antimicrobials, morbidity and mortality of S. aureus endovascular infections remains high because of the rapid local tissue destruction and the release of exotoxins during fulminant sepsis. Prevention is the best solution, and this requires a better understanding of pathogenic mechanisms for development of S. aureus endovascular infections.

Mature von Willebrand factor (VWF) is a large multifunctional glycoprotein containing 2050 amino acid residues (subunit size, ~250–270 kDa) consisting of four types of repeats (designated A–D), each consisting of 3 or 4 domains [13]. The essential role of the protein in hemostasis [14] consists of transport and stabilization of coagulation factor VIII [15] and support of platelet adhesion to subendothelial tissue at sites of vascular injury, particularly in the presence of high shear stress [16, 17], as present in capillaries, on heart valves, or at vessel bifurcations [18]. To promote platelet attachment to subendothelium, pro-VWF, consisting of the mature subunit and a 741-residue propeptide, must form dimers through disulfide linkages near the carboxy-terminus [19] and is subsequently multimerized to molecules of up to 15 million Da size [20, 21] through disulfide bonds near the amino-termini [22]. Subsequently, the propeptide is cleaved. Both platelets (in α-granules) and endothelial cells (in Weibel-Palade bodies) store VWF, and the protein is released either constitutively by endothelial cells or by stimulated release from both platelets and endothelial cells. Released VWF is characterized by the presence of high-molecular-weight multimers [21], which are subsequently cleaved in plasma by proteases [23, 24], resulting in heterogeneity of plasma multimers; this VWF binds to various subendothelial components, such as collagens type I and type III, proteoglycans, heparin-like glucosaminoglycans, and sulfatides [14, 25]. Platelets interact with VWF through two distinct receptors: GPIb in the GPIb-IX-V complex and the GPIIb-IIIa complex [26], with GPIb mediating the initial contact and GPIIb-IIIa recognizing VWF after platelet activation [27]. Both receptors are essential for shear-induced platelet aggregation at sites of high wall shear rate. Binding occurs through the A1 domain recognized by GPIb [28] and the adhesion-mediating Arg-Gly-Asp-Ser motif in the C1 domain near the carboxy-terminus of VWF recognized by activated GPIIb-IIIa [29]. The multimeric structure of VWF is essential for proper functioning in platelet adhesion to subendothelium, as evidenced by qualitative variants of VWF (e.g., type IIA von Willebrand disease) resulting in bleeding disorder [30] and by in vitro demonstration of the effect of various VWF multimers on adhesion of platelets to subendothelium [31]. Thus, multimeric VWF of various sizes is likely to be present at sites of endothelial cell damage prone to staphylococcal infection, such as altered endocardium.
In the vascular system, stable bacterial adhesion requires both attachment and resistance to detachment under shear conditions. We hypothesized that immobilized VWF may, similar to the interaction with blood platelets, serve as an adhesive substrate for circulating S. aureus. Therefore, as a first step, we evaluated binding characteristics of soluble VWF to S. aureus as well as promotion of S. aureus adhesion to VWF-adsorbed surfaces.

Materials and Methods

Bacteria. Binding and surface adhesion experiments were performed with S. aureus Cowan 1. In addition, binding experiments were performed with S. aureus strains 8325-4, Newman, SA 113, and 6850 [32] and a variety of clinical bacteremic isolates from patients with foreign body infection as described [33].

Purification of VWF. VWF was purified as previously described [34] by use of Haemate-HS500 cryoprecipitates (supplied by Behring, Marburg, Germany). Size chromatography was performed by use of a gel filtration column containing BioGel A-15m (Bio-Rad, Richmond, CA) as bed matrix. For matrix equilibration and protein elution, a gel filtration buffer containing TRIS-HCl (50 mM, pH 7.35) (Sigma, St. Louis), NaCl (150 mM), and Na2 citrate (5mM) (Merek, Darmstadt, Germany) was used. For prevention of proteolytic degradation of VWF multimers [23], a proteinase-inhibitor cocktail containing PMSF (Sigma; final concentration, 1 mM), leupeptin hemisulfate (5 μM [Sigma]), and aprotinin (10 μM [Trasyol; Bayer, Leverkusen, Germany]) was added. Fractions of 1.5 mL were collected, and concentration and activity of VWF eluted was estimated by use of the BCA protein assay (Pierce, Rockford, IL), a VWF ELISA [35], and an assay for ristocetin cofactor activity [36], respectively. The first three fractions containing high-molecular-weight VWF were used for further experiments. Purity was checked with both Coomassie blue – and silver-stained SDS-PAGE according to standard methods. Copurified trace amounts of fibrinogen were quantitated by ELISA with polyclonal anti-fibrinogen antibodies (DAKO Diagnostic, Hamburg, Germany) calibrated with appropriate dilutions of normal citrated plasma (containing 2.5 mg/mL fibrinogen). Multimerized VWF was resolved under nonreduced conditions as described [37] with the PhastSystem (Pharmacia Biotech, Freiburg, Germany) using 0.8% agarose and diffusion blotting on nitrocellulose. Transferrred proteins were probed with anti-VWF antibodies (DAKO) conjugated with alkaline phosphatase. Alternatively, recombinant VWF was expressed in baby hamster kidney cells according to [38]. The recombinant VWF has previously been shown to multimerize in a manner similar to plasma VWF [39].

Labeling of VWF. Iodobeads (Pierce) were prepared according to manufacturer’s instructions. Purified VWF (750 μL; 150 μg/ mL) was incubated with 200 μCi of 125I (Amersham Buchler, Braunschweig, Germany) and 1 Iodobead for 3 min at room temperature. Unbound iodine was removed by dialysis tubings (cutoff, 14 kDa) pretreated with hexadimethrin bromide (Polybrene [Fluka, Neu-Ulm, Germany]) against a buffer containing TRIS-HCl (50 mM), NaCl (150 mM), and Na2-citrate (5 mM). More than 95% of iodine was found to be protein-bound as determined by trichloroacetic acid–precipitable counts per minute. The specific activity of the iodinated protein was 200,000 cpm/μg. Purity of the labeled protein was checked by use of overexposed autoradiographs of SDS-PAGE.

Extraction assay. Unlabeled purified VWF (4 μg/mL) was incubated with increasing (vol/vol) concentrations of S. aureus cells (1 h, 37°C). The incubation mixture was then centrifuged, and the concentration of VWF antigen (U/mL) in the supernatant was determined by quantitative immunoelectrophoresis with a rabbit polyclonal antibody to human VWF as previously described [40] and subsequently modified by use of a radiolabeled anti-VWF monoclonal antibody, AvW-1 [41].

Binding assay. S. aureus strains (2 × 108 cfu each) from a fresh overnight culture grown in brain-heart infusion media were briefly sonicated (10 cycles of 1 s, 50 W; Branson, Danbury, CT) and incubated with 2 μL of diluted (20,000 cpm/μL) 125I-labeled VWF (60 min, 20°C) in PBS containing 1 mM Ca2+ and 0.5 mM Mg2+ supplemented with Tween 20 (0.05%; PBST) and bovine serum albumin (0.1%; BSA) in a total volume of 0.2 mL. The reaction was stopped with 1 mL of ice-cold PBST, bacteria were washed twice (12,000 g, 5 min), and counts in the pellet were determined. Alternatively, 2 × 108 cfu of S. aureus Cowan 1 was incubated with varying quantities of 125I-labeled VWF (2–48.5 μg/mL) in BSA-PBST, washed, and evaluated for bound counts per minute. Binding inhibition was determined by using two different approaches. In dose-response kinetic studies (total volume, 0.5 mL), S. aureus cells (2 × 108 cfu/mL) were preincubated with various concentrations of unlabeled VWF (0–24 μg/mL); then 125I-labeled VWF (0.5 μg/mL) was added. The incubation mixtures were further incubated (60 min), and unbound ligand was removed by centrifugation. In time-course studies, S. aureus cells were incubated with either 125I-labeled VWF (15 μg/mL) or 125I-labeled VWF (15 μg/mL) supplemented with 15 μg/mL unlabeled VWF (total volume, 3 mL), and after various time intervals, aliquots were removed and evaluated for bound 125I-labeled VWF.

Adsorption of VWF to polymethylmethacrylate (PMMA) and adhesion of S. aureus to VWF-adsorbed PMMA. PMMA was used as the model surface, because it has been previously studied as a standard solid interface in adhesion experiments with staphylococci [33, 42–45] and because of its clinical applications. For evaluation of protein adsorption, 40-μL quantities of various concentrations of 125I-labeled VWF in PBS were layered on top of PMMA coverslips (size, 7 × 7 mm) as previously described [45] and incubated in a humid chamber (30 min, 37°C). Thereafter, coverslips were washed twice in PBS, and surface-adsorbed radioactivity was determined. For evaluation of adhesion of S. aureus to VWF-adsorbed surfaces, 40-μL quantities of a solution containing various concentrations of unlabeled, native, multimeric plasma VWF (range, 0–64 μg/mL) or recombinant VWF were adsorbed onto PMMA coverslips (1 h, 37°C). Thereafter, coverslips were washed and transferred into petri tubes. For radiolabeling, S. aureus organisms were grown for 4 h in Mueller-Hinton broth supplemented with [3H]thymidine as previously described [43], then washed twice and sonicated. Coverslips were incubated with 1 mL of a solution containing staphylococci (4 × 106 cfu/mL), human serum albumin (0.5%), and Ca2+/Mg2+-PBS in a stirred water bath (60 min, 37°C). Thereafter, fluids were decanted, coverslips with adherent bacteria were washed twice, and adherent radioactivity was determined.
Results

Purified VWF was resolved on 7.5% SDS-polyacrylamide gels and evaluated with either Coomassie blue- or silver-stained SDS-PAGE. As shown in figure 1 (lane a), under reduced conditions, VWF migrated at 250–270 kDa, and the preparation was >99% pure without significant proteolysis. Copurification of fibrinogen was found by ELISA to be at most 1.1 µg/mL (0.31% of VWF concentration). The protein concentration obtained ranged between 150 and 360 µg/mL, with a ristocetin-cofactor activity of 15–40 U/mL. In Western blot experiments using polyclonal anti-VWF antibodies (DAKO), VWF appeared as a single band (figure 1 [lane b]), whereas probing with polyclonal anti-fibrinogen and anti-thrombospondin antibodies did not yield a positive signal (not shown). Purity of VWF was also checked by overexposed autoradiography of the 125I-labeled protein without demonstration of additional bands (not shown). Recombinant VWF (30 µg/mL) migrated at a similar position in the gel (figure 1 [lane c]). As previously shown using mercaptoethanol reduction gradient [23], large-molecular-weight plasma VWF fractions vary between 20 and 40 multimers [25]. While we did not perform reduction gradient analysis for exact determination of the VWF size in our plasma preparation, it was highly multimerized, as demonstrated by use of PhastSystem chromatography under nonreduced conditions in agarose gels (figure 1 [lane d]).

VWF binding to S. aureus Cowan 1 was determined with an extraction assay by incubating S. aureus cells (up to 80% [vol/vol], corresponding to 2.5 × 10^11 cfu/mL) with VWF [4 µg/mL]. After 1 h of incubation and subsequent centrifugation, the concentration of VWF antigen in supernatants decreased as the S. aureus cell concentration increased (figure 2), and in supernatants of assays containing 70% (vol/vol) S. aureus cells, only 27.1% (0.19 U/mL) of the VWF antigen concentration present in tubes without S. aureus cells (0.7 U/mL) was found. For binding studies, various concentrations of 125I-labeled VWF were incubated with 2 × 10^8 cfu of S. aureus Cowan 1. After removal of unbound ligand by centrifugation, bound counts per minute in the pellet were determined. Kinetics of VWF binding to S. aureus Cowan 1 revealed a dose-dependent binding reaction without clear demonstration of saturation over the tested dose range (up to 48.5 µg/mL) (figure 3). For determination of binding inhibition, S. aureus Cowan 1 (2 × 10^8 cfu/mL) was preincubated with unlabeled VWF using varying concentrations of up to 24 µg/mL (37°C, 30 min). Then, 0.5 µg/mL 125I-labeled VWF was added, reaction mixtures were further incubated (37°C, 60 min), and unbound ligand was removed by centrifugation. Under these conditions, no appreciable inhibition of binding was obtained (data not shown). 125I-labeled VWF bound not only to S. aureus Cowan 1 but also to S. aureus strains 8325-4, Newman, 6850, and 113 and to 5 blood isolates from patients with foreign body infection (figure 4). Time courses of binding revealed a rapid interaction of 125I-labeled VWF with S. aureus Cowan 1 cells, with maximal binding present after 5 min of incubation and remaining constant for up to 120 min of incubation.

As shown in figure 5, adsorption of 125I-labeled purified VWF to PMMA coverslips increased in a dose-dependent manner and reached a plateau at ~400 ng/cm² VWF adsorbed.
Figure 3. Binding assay of VWF to S. aureus Cowan 1. Indicated concentrations of $^{125}$I-labeled VWF were incubated with $2 \times 10^8$ cfu S. aureus Cowan 1 (60 min, 20°C) in 200 $\mu$L of PBS containing Ca$^{2+}$, Mg$^{2+}$, Tween 20 (0.05%), and bovine serum albumin (0.1%). After binding, bacteria were sedimented (3000 g, 5 min), and counts in pellet were determined. Shown are means ± SEs of 3 experiments (duplicate determination).

Figure 4. Binding assay of VWF to various clinical and laboratory S. aureus isolates. Binding conditions were as shown in figure 3. Bacteria were incubated with 40,000 cpm of $^{125}$I-labeled VWF. Results are means of triplicate determination ± SEs.

Figure 5. Adsorption of $^{125}$I-labeled VWF to polymethylmethacrylate (PMMA) coverslips; 40 $\mu$L PBS containing indicated concentrations of radiolabeled VWF was deposited on coverslip surfaces (30 min, 37°C). After washing, adsorbed VWF was determined by measuring coverslip-adherent counts. Results are means ± SEs (triplicate determination).

Figure 6. Promotion of adhesion of S. aureus Cowan 1 to VWF-adsorbed polymethylmethacrylate (PMMA). [$^{3}$H]thymidine-labeled S. aureus Cowan 1 ($4 \times 10^6$ cfu) was incubated with coverslips preadsorbed with indicated concentrations of unlabeled VWF. After incubation, coverslips were washed twice, and PMMA-adherent counts were determined. Results are means of 2 experiments in triplicate.

Corresponding to $\sim 0.4 \mu g/cm^2$ (equaling $5 \times 10^{10}$ multimerized VWF molecules/cm$^2$ of PMMA when assuming an average of 10 VWF polypeptide chains/molecule). For determination of adhesion of S. aureus to immobilized VWF, coverslips were preadsorbed with various concentrations of VWF, then incubated with $^{3}$H-labeled S. aureus Cowan 1 ($4 \times 10^6$ cfu/assay) in albumin-containing buffer and evaluated for adherent bacteria. In the absence of VWF, staphylococcal adhesion was low, with only $1.2 \times 10^3$ cfu (17 cpm). Adhesion increased as a function of the concentration of VWF used to coat PMMA coverslips, paralleled VWF adsorption (with a linear increase between 5 and 30 $\mu g/mL$ VWF), and showed partial saturation at higher concentrations of VWF used for adsorption. At 64 $\mu g/mL$ VWF, the increase of staphylococcal adhesion over adhesion without VWF was 67-fold (figure 6). Similar results were obtained if recombinant VWF was used instead of VWF purified by gel chromatography. On preadsorption of PMMA with recombinant VWF (30 $\mu g/mL$), $2.9 \times 10^4 \pm 1.5 \times 10^4$ cfu (mean ± SE, n = 3) of S. aureus Cowan 1 adhered to
the coverslips (fold increase, 27.1). In some experiments, ³H-labeled *S. aureus* Cowan 1 was treated with bovine trypsin (specific activity, 10,000 U/mg [Sigma], 15 min, 37°C), then with soybean trypsin inhibitor (type II-S [Sigma], 15 min, 37°C), and washed. While adhesion of untreated *S. aureus* Cowan 1 to VWF-adsorbed PMMA (30 µg/mL) was 1.48% ± 0.18% (mean ± SE, n = 5), after treatment with 0.05 mg/mL, trypsin adhesion decreased to 0.88% ± 0.17%, and after treatment with 1 mg/mL, trypsin adhesion was 0.40% ± 0.08% (P = .04 and .002, respectively, vs. control). In addition to *S. aureus* strain Cowan 1, adhesion of *S. aureus* strains Newman, 8325-4, 113, and 6850 as well as of the clinical bloodstream [56], thrombospondin [45], and laminin [57]) should result in an aureus strain, adhesion of S. aureus to these strains, 6850 being least extensively promoted (figure 7). In contrast to these strains, *S. aureus* Wood 46 (a protein A-negative strain) did not appreciably bind to adsorbed VWF.

**Discussion**

Intravascularly circulating *S. aureus* have a predilection for exposed subendothelial stroma [46] and for prosthetic material [47]. These sites are coated with cellular or proteinaceous host factors. Hence, an analysis of pathogenic mechanisms contributing to initial adhesion of staphylococci must take into account the distribution and conformation of the specific host ligand in tissue or on prosthetic material, the characteristics of binding of the host factor to the microorganism and its ability to promote microbial surface adhesion, and the physiologic conditions under which the interaction is likely to occur. With this approach, a variety of proteins and cell types important for staphylococcal adhesion to a substratum have been identified (reviewed in [48]), and, by use of defined mutants deficient in one or several microbial adhesins, a role in pathogenicity of some of these ligands [49, 50] and of several genetic regulatory elements [11, 51, 52] has been shown.

In this study, we have identified a novel candidate host ligand for adhesion of *S. aureus* to intravascular sites, VWF. Adsorption kinetics of VWF to PMMA paralleled those previously described for fibronectin [43] and fibrinogen (to polybutylmethacrylate) [53]; that is, they showed saturable adsorption kinetics, reaching a plateau at concentrations of ≥100 µg/mL soluble protein. The plateau concentration of VWF adsorbed on the surface (0.4 µg/cm²) was similar to the respective concentrations described for fibronectin (0.32 µg/cm²) or thrombospondin (1.5 µg/cm²) [45]. These adsorption kinetics follow a model of saturation kinetics derived from the Langmuir type of adsorption isotherm and result in saturable adhesion of bacteria [43, 45] or cells [53] to the precoated surfaces. Thus, whereas VWF is present only in small amounts in plasma (7 µg/mL) [20], it is present at sites of subendothelial matrix exposure depending on endothelial cell or platelet activation [21, 25] and has been shown (together with collagen) to be instrumental in adhesion of platelets to atherosclerotic coronary arteries [54]. Our data showing avid adsorption to biomedical polymers suggest that it may be present on implanted or inserted polymeric surfaces at sites of platelet activation and α-granule release.

Binding of VWF in suspension was characterized by a linear dose-relationship of binding without clear saturation at concentrations up to 50 µg/mL. Taking into account the size of the multimeric protein, binding affinity with *Kₐ* values in the order of nanomolars (as described for fibronectin [55], fibrinogen [56], thrombospondin [45], and laminin [57]) should result in 10–20 times higher VWF concentrations necessary for half-maximal binding (equaling *Kₐ*) compared with these proteins. Thus, while in binding assays, protein concentrations as high as 100 µg/mL and above may be required to demonstrate saturation, these concentrations are difficult to evaluate because of formation of protein-protein complexes yielding increased background values and because of multiple protein-bacteria interactions. Furthermore, binding of large glycoproteins to gram-positive cell surfaces may not necessarily exhibit all characteristics indicative of an equilibrium process; for example, the fibronectin–*S. aureus* interaction, although saturable, becomes irreversible immediately after initial interaction [55]. In other systems, this type of ligand-receptor behavior may indicate a conformational change of the ligand [58]. In addition, as shown for streptococci [59], bacteria may interact with host cells or adhesive proteins through multiple binding sites and may therefore not display binding kinetics typical for small molecules and singular receptors. Therefore, whereas our data do not unequivocally indicate a receptor-mediated binding of VWF to *S. aureus*, they do not exclude the presence of specific
The determination of ligand binding to microorganisms in suspension is of importance, demonstration of the ability of the protein to promote bacterial adhesion to solid surfaces is essential for attributing to it a pathogenic role in endovascular infection. On adsorption, surfaces can induce conformational changes in adsorbed proteins such as fibrinogen [53] or fibronectin [60], further influencing interaction of bacteria with the adsorbed ligand, for example by interaction of bacteria with an alternative site on the protein ligand not recognized in suspension systems [61]. Several in vitro studies have shown that *S. aureus* attachment to polymeric surfaces is strongly promoted by selectively adsorbed plasma or extracellular matrix proteins such as fibrinogen, fibronectin, collagen, vitronectin, laminin, thrombospondin, bone sialoprotein, or elastin (reviewed in [62, 63]). In this study, VWF could be shown to promote adhesion of *S. aureus* Cowan 1 as well as adhesion of other clinical and laboratory staphylococcal strains. Adhesion appears to be promoted to a similar extent by surface-adsorbed VWF compared with thrombospondin [45] and slightly less compared with fibronectin or fibrinogen [33]. Our data showing a strong reduction in bacterial adhesion to VWF-PMMA after trypsin treatment of cells may suggest the protein nature of these experiments may yield further information on the including experiments under defined shear forces [75] and ating pathogenic mechanisms. The findings presented in our have been instrumental in targeting adhesive proteins with and nucleotide sequence of a man into the endovascular milieu, the physiology of plasma tion of the clumping factor (*β*-fibrinogen receptor) of

Particular attention was given to avoid copurification of other proteins that could have influenced the results with VWF. When size chromatography is used for purification of VWF, copurification with trace amounts of fibrinogen is a recognized problem; however, for binding studies (such as those involving thrombin-activated platelets), the quantity of fibrinogen present in the early fractions of VWF preparations appears to be neglig-ible and requires sensitive detection techniques [26]. Moreover, apart from absence of contaminating proteins as determined by use of standard techniques, our data obtained with recombinant VWF make an influence of adhesive proteins co-purified with VWF in our binding or adhesion assays highly unlikely. Thus, our results indicate that VWF binds to *S. aureus* and that surface-adsorbed VWF may contribute to colonization of endovascular substrata with staphylococci.

After endothelial cell damage or insertion of polymer mater-ial into the endovascular milieu, the physiology of plasma protein-exposed surfaces is highly complex, because of a large array of differentially adsorbed and desorbed proteins and cells. Clinical studies using inserted catheters harvested from patients [65, 66] or using ex vivo vascular shunt material from animals [67] have been instrumental in targeting adhesive proteins with a key role in early staphylococcal adhesion. Other proteins also may play a role in staphylococcal pathogenesis due to their unique spatial and temporal distribution at sites of local coagu-lation [45], cell activation [68, 69], or subendothelial matrix exposure [33, 70–73]. While these conditions may vary widely, the presence of blood flow and resulting shear stress during microbial adhesion is ubiquitous throughout the vascular sys-tem. The unique properties of multimerized VWF in promoting platelet adhesion in the presence of shear, together with our findings of VWF binding to *S. aureus* and promotion of staphylococcal adhesion to surfaces, opens new perspectives for the characterization of a ligand potentially essential for the coloni-ization of endovascular surfaces during blood flow. Our findings also shed new light on the observation that pigs with von Willebrand disease appear to be resistant to streptococcal experi-mental endocarditis [74], because it is conceivable that in the absence of functional VWF in autosomal recessive porcine von Willebrand disease, microbial adhesion to damaged heart valves may not be supported. However, these observations must be considered preliminary because of a small number of ani-mals tested, and they do not provide clarification of the underly-ing pathogenic mechanisms. The findings presented in our study may now open new directions for further research, including experiments under defined shear forces [75] and attemps to identify staphylococcal adhesins recognizing VWF. These experiments may yield further information on the importance of the *S. aureus*–VWF interaction in vivo and, by identi-fying adhesins that may be blocked by an immunotherapeutic approach, may provide the basis for interfering in early steps of *S. aureus* endovascular infections.

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References


