Supplemental Data

Adhesion of Staphylococcus aureus to the vessel wall under flow is mediated by von Willebrand factor-binding protein

Short title: vWbp mediates S. aureus adhesion under flow

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Supplementary Materials and Methods

Bacterial strains

All strains were stored in Brain Heart Infusion (BHI) with 10% glycerol at -80°C. Bacteria were grown overnight in tryptic soy broth (TSB) at room temperature (RT). For fluorescent labeling, bacteria were washed with PBS (phosphate buffered saline, Gibco, Life Technologies, Belgium) and stained with 5(6)-carboxy-fluorescein N-hydroxysuccinimidyl ester (Sigma-Aldrich, Germany) (final concentration 30 µg/mL for perfusion experiments and 60 µg/mL for *in vivo* experiments). Optical densities of bacterial suspensions were adjusted to an OD$_{600}$ of 0.65 or 1.2 (corresponding to approximately $3 \times 10^8$ and $6 \times 10^8$ colony forming units (CFU)/mL for *in vitro* experiments) and 1.8 (corresponding to approximately $1 \times 10^9$ CFU/mL for *in vivo* experiments) in Dulbecco’s modified eagle medium (DMEM) (Life Technologies, Belgium). Bacterial suspensions were further diluted (1:1) in platelet-poor plasma (PPP) or platelet-rich plasma (PRP).

Preparation of PRP

Blood was drawn from the antecubital vein of consenting healthy non-medicated volunteers into 1/10 vol/vol of 3.8% sodium citrate. Citrated whole blood was used for flow chamber experiments. PRP was obtained by centrifugation of citrate-treated blood samples (15 min, 150 g) with platelet counts adjusted to $2.5 \times 10^5$ platelets/µL using autologous PPP. PRP was spiked with rhodamine-G (1 µg/mL) (Invitrogen, Life Technologies, Belgium).

Static experiments

Twelve-well plates were coated with VWF (50 µg/mL) at RT for 4 hours and blocked with 1% bovine serum albumin (BSA, Sigma-Aldrich, Germany). Labeled WT and *vwb* strains were added (OD$_{600}$ 0.65), incubated on 37°C for 30 minutes and washed 3 times with PBS. Live images were obtained and recorded as described before. Correction for background data was performed by subtracting bacterial binding to BSA coating.
Bacterial adhesion to ECs

Human umbilical vein endothelial cells (HUVECs), isolated from fresh umbilical cords of healthy donors, were mobilized by 0.2% collagenase type 1 (Gibco, Life Technologies, Belgium) and grown to a confluent monolayer in 6-well plates (Greiner Bio-One, Germany) in EGM-2 Bullet Kit + Single Quots (Lonza, Switzerland). Isolated primary HUVECs were seeded on gelatin-coated plastic coverslips (Sarstedt, Germany) and grown to 70-80% confluency in a CO₂-incubator at 37°C. The coverslips were mounted in a micro-parallel flow chamber and perfusions were carried out with an infusion pomp generating a shear rate of 1000 s⁻¹.

Scanning electron microscopy (SEM)

After perfusion with bacteria as described above, the coverslips were fixed overnight at 4°C in 0.1 M sodium-cacodylate buffer (Sigma-Aldrich, Germany) containing 2.5% glutaraldehyde. After washing, samples were additionally post-fixed for 2 hours at 4°C with 1% osmium tetroxide (Sigma-Aldrich, Germany) and dehydrated through a graded ethanol series. Samples were transferred to hexamethyl disilazane (HMDS, Sigma-Aldrich, Germany) for 15 minutes and dried overnight in a critical point dryer (CPD7501, Polaron, UK). Mounted samples were coated with platinum and pictures were taken using a field emission scanning electron microscope (JSM-7401F, JEOL, Akishima, Japan).
Supplementary figures

Supplementary Figure 1. Shear-dependent adhesion of *S. aureus* to VWF and collagen. (A) 12-well plates were coated with VWF (50 µg/mL) and blocked with 1% BSA. Fluorescently labeled WT and *vwb* strains were added and the well plates were incubated on 37°C for 30 minutes. Adhesion to VWF in static conditions is low, and did not differ between WT and *vWbp*-deficient strain (n≥7). (B) Micro-parallel flow chamber perfusion over coated VWF with WT strain at shear rates of 250, 500, 1000 and 2000 s⁻¹. Adhesion of the WT strain to VWF increased with increasing shear stress (n≥4). (C) Perfusion over coated collagen with WT strain at shear rates of 250, 500, 1000 and 2000 s⁻¹. VWF (60 µg/ml) was added where indicated (n≥4). All results are expressed as mean±SEM. * P < 0.05, ** P < 0.01.
Supplementary Figure 2. Reconstitution of vWbp expression restores the adhesive phenotype. (A) Micro-parallel flow chamber perfusion over coated VWF (50 µg/mL) with fluorescently labeled WT, coa/vwb and coa/vwb + plasmid at a shear rate of 1000 s⁻¹. (B) Perfusion over coated collagen with WT, coa/vwb and coa/vwb + plasmid at a shear rate of 1000 s⁻¹. VWF (60 µg/mL) was present in the perfusate where indicated. Insertion of the plasmid rescued the reduction in adhesion to VWF of the Coa- and vWbp-deficient mutant strain, as well as the adhesion to collagen in the presence of VWF. No significant differences were observed between the WT strain and coa/vwb + plasmid strain. All results are expressed as mean±SEM. ** P < 0.01, n≥4.

A  Adhesion of coa/vwb to VWF is rescued by the Coa/vWbp plasmid

B  Adhesion of coa/vwb to collagen in the presence of VWF is rescued by the Coa/vWbp plasmid
Supplementary Figure 3. Effect of exogenous vWbp on *S. aureus* adhesion. (A-B) Perfusion over coated collagen with fluorescently labeled WT or vwb strains and 60 µg/ml VWF at a shear rate of 1000 s⁻¹. Addition of His₆-vWbp (15 µg/ml) and supernatants of WT or vwb strains where indicated (n≥4). (C) Perfusions over coated VWF (50 µg/mL) with vwb at a shear rate of 1000 s⁻¹. Different concentrations of His₆-vWbp were added as indicated (n≥4). (D) Differential effects of His₆-vWbp preperfusion on the adhesion of WT and vwb strains. Preperfusion over coated VWF with His₆-tagged vWbp (final concentration 15 µg/ml, 10 minutes) followed by perfusion with *S. aureus* at 1000 s⁻¹ increased adhesion of the vwb strain but blocked adhesion of the WT strain (n≥4). All results are expressed as mean ± SEM. * P < 0.05, *** P < 0.001.
Supplementary Figure 4. vWbp coagulase activity enhances bacterial adhesion to collagen. Micro-parallel flow chamber perfusion over coated collagen with fluorescently labeled coa strain at 1000 s\(^{-1}\) after preincubation in plasma for the indicated time-intervals. vWbp coagulase activity during the preincubation phase increased subsequent adhesion in a time-dependent way. Addition of dabigatran (final concentration 500 nM) where indicated (n\(\geq\)5). All results are expressed as mean ± SEM. * \(P < 0.05\), ** \(P < 0.01\) *** \(P < 0.001\).
Video 1-2. Preincubation of *S. aureus* Newman in plasma increases bacterial adhesion to collagen.

Micro-parallel flow chamber perfusion over coated collagen with fluorescently labeled WT strain in plasma with (2) or without (1) preincubation (37°C for 15 minutes) with a shear rate of 1000 s⁻¹. Via video-microscopy, time-laps images were acquired using a black and white camera (Carl-Zeiss AxioCam MRm) under an inverted inverted microscope (Axia-observer D1, Carl-Zeiss NV, Zaventem, Belgium) with 20x objective lens.


Video 4. Real-time adhesion of *S. aureus* Newman to activated vessel wall in Vwf⁺/⁻ mice.

Video 5. Real-time adhesion of the vwb strain to activated vessel wall in Vwf⁺/⁻ mice.

Video 6. Real-time adhesion of *S. aureus* Newman to activated vessel wall in Vwf⁻/⁻ mice.

*In vivo* venous mesenteric perfusion model with C57Bl/6-Vwf⁺/⁻ and Vwf⁻/⁻ mice. Five µL of the Ca²⁺-ionophore A23187 (10 mM) was applied to the region of the visualized vascular bed to trigger ECs activation and VWF-release. A suspension of carboxy-fluorescein-labeled *S. aureus* Newman was injected through the jugular catheter. The mesenteric circulation was visualized under an inverted microscope (Axia-observer D1, Carl-Zeiss NV, Zaventem, Belgium) with 20x objective lens. Via video-microscopy, time-laps images were acquired using a black and white camera (Carl-Zeiss AxioCam MRm).