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**Clumping factor A is a membrane receptor for secreted von Willebrand factor-binding protein mediating Staphylococcus aureus adhesion to vascular endothelium**

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Staphylococcus aureus (S. aureus) is a frequent pathogen causing life-threatening endovascular infections such as infective endocarditis. To establish an endovascular infection, S. aureus needs to bind to vascular von Willebrand factor (VWF) to overcome shear forces imposed by flowing blood. We previously described that secreted staphylococcal von Willebrand factor-binding protein (vWbp) interacts with VWF, enabling flow-controlled bacterial adhesion to endothelial cells (ECs) and to subendothelial matrix. However, the receptor to which vWbp anchors to the bacterial cell wall is unknown so far. Several surface proteins of S. aureus are linked to the bacterial cell wall by sortase A (SrtA). A mutation in the srtA gene leads to an anchoring defect of about 20 S. aureus surface proteins. We hypothesized that vWbp is able to interact with a staphylococcal surface protein, thus mediating the adhesion of S. aureus to ECs and to subendothelial matrix via VWF.

We measured adhesion of S. aureus Newman (WT) and mutants deficient in SrtA or in SrtA-dependent surface proteins to VWF, vWbp and ECs. Fluorescently labeled bacteria were perfused over a glass surface coated with VWF, vWbp or ECs in a micro-parallel flow chamber. We verified our findings using Lactococcus lactis (L. lactis) bacteria expressing single staphylococcal surface proteins. In vivo adhesion was evaluated in the murine mesenteric circulation using real-time intravital vascular microscopy.

First we quantified the adhesion to coated vWbp of several S. aureus strains deficient in individual surface proteins or deficient in SrtA. Compared to the WT strain, the SrtA deficient strain and the strain deficient in Clumping factor A (ClfA) showed a decreased adhesion to vWbp. The absence of ClfA also reduced the adhesion of S. aureus to VWF under flow, to activated ECs and to the activated murine vessel wall in vivo. Selective overexpression of ClfA in the membrane of L. lactis enabled the bacteria to bind to coated vWbp under flow.

We conclude that vWbp interacts both with sheared VWF and with the SrtA-dependent staphylococcal surface protein ClfA. The ternary complex formed by endothelial VWF, secreted vWbp and bacterial ClfA causally mediates adhesion of S. aureus to the vascular endothelium.