Expression of Pls (Plasmin Sensitive) in \textit{Staphylococcus aureus} Negative for \textit{pls} Reduces Adherence and Cellular Invasion and Acts by Steric Hindrance

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\textbf{Background.} The methicillin-resistant \textit{Staphylococcus aureus} (MRSA) surface protein Pls (plasmin sensitive) reduces adhesion to host proteins and cellular invasiveness by an unknown mechanism that requires Pls expression. Here, we tested the effect of Pls expression using different \textit{pls}-negative backgrounds.

\textbf{Methods.} Three \textit{pls}-negative strains (the methicillin-susceptible \textit{Staphylococcus aureus} strains Cowan I and 6850 and the MRSA strain ST239-635/93, which harbors staphylococcal cassette chromosome [SCC] mec type III) were transformed. Constructs used were full-length \textit{pls} (pPLS4), \textit{pls}-\textit{D}LPDTG (no sortase motif; pPLS5), and \textit{pls}-\textit{DS} (no serine–aspartic acid [SD] repeats; pPLS6). Adherence, invasiveness, gene expression, and surface expression were quantified by photometry, flow cytometry, real-time reverse-transcription polymerase chain reaction, and a modified enzyme-linked immunosorbent assay, respectively.

\textbf{Results.} In Pls-expressing strains (those with pPLS4), adherence to immobilized fibronectin (Fn) and binding of soluble Fn was reduced by \(~20\%\) and \(~25\%\), respectively. Invasion of 293 cells and EA.hy 926 cells was reduced by up to 85\%. Surprisingly, transcription of \textit{fnbA} and \textit{spa} was up-regulated, but transcription of \textit{clfA} and \textit{hla} was down-regulated. Pls and Fn-binding protein (FnBP) surface expression was increased. Competition with purified FnBPA, but not with Pls, reduced invasiveness by \(~90\%\). The invasiveness of 6850 (pPLS5) and of 6850 (pPLS6) was reduced by only \(~20\%\) and \(~15\%\), respectively.

\textbf{Conclusion.} Expression of cell wall–anchored Pls reduces adherence and invasiveness independently of the MRSA/SCCmec background. This occurs despite early up-regulation of \textit{fnbA} transcription and FnBP surface expression. Thus, Pls acts by steric hindrance rather than another mechanism.

\textit{Staphylococcus aureus} causes serious community-acquired and nosocomial infections that result in a broad spectrum of diseases, such as boils, wound infection, endocarditis, osteomyelitis, and sepsis or septic shock. Treatment of infection is complicated by the emergence of methicillin-resistant \textit{S. aureus} (MRSA) strains, some of which—especially those belonging to staphylococcal chromosomal cassette (SCC) mec types I–III—have also acquired multidrug resistance. Adherence of \textit{S. aureus} to host molecules is a prerequisite for tissue colonization and overt disease. \textit{S. aureus} expresses a large number of cell-surface proteins (adhesins) that adhere to host glycoproteins, and some adhesins (i.e., fibronectin [Fn]–binding proteins [FnBPs] and extracellular adherence protein) also have invasive functions.
Well-characterized surface proteins include immunoglobulin (Ig) G–Fc–binding [1, 2] and von Willebrand factor–binding [3] protein A (SpA); FnBPA and FnBPB [4–7]; fibrinogen (Fg)–binding clumping factor (Clf) A, ClfB, and extracellular Fg-binding protein [8–10]; collagen adhesin [11]; elastin-binding protein A of S. aureus [12]; and bone sialoprotein–binding protein [13]. An additional MRSA surface protein is sensitive to proteolysis by plasmin and trypsin and is thus called Pls (plasmin sensitive). Similarly, the activation of receptor-bound host plasminogen to plasmin on the S. aureus surface [14] leads to cleavage of the intact 230-kDa Pls protein into 175- and 68-kDa fragments (apparent molecular masses) [15]. The pls gene is located in SCCmec type I [16, 17]. SCCmec also contains the mecA gene, which encodes a modified penicillin-binding protein 2. We have recently identified a pls-positive SCCmec type IV strain [18]. Pls is a homologue of the serine–aspartic acid (SD) repeat–containing (Sdr) family of surface proteins [19], of which ClfA is the best-characterized member (reviewed in [20]). Parts of Pls are homologous to accumulation-associated protein (Aap) of Staphylococcus epidermidis, which is essential for biofilm formation [21]. Addition of purified Pls to a MRSA suspension leads to bacterial aggregation, suggesting that Pls plays a role in bacterial cell-cell interactions [22]. S. aureus surface protein G, a recently described partial S. aureus homologue of Aap and Pls [23], has been reported to mediate binding to human desquamated nasal epithelial cells [24].

We have previously shown that the presence of Pls decreases the adherence of MRSA strains to immobilized Fn, Fg, IgG, and laminin as well as to soluble Fn and Fg [17]. Furthermore, we have shown that expression of Pls also decreases the cellular invasiveness of MRSA isolates [25] and that a pls-negative mutant adheres better to immobilized Fn, Fg, and IgG than does its parental strain [17, 25]. Overall, pls-positive MRSA isolates are less adherent and invasive than pls-negative MRSA isolates [18, 25]. Pls expression is required for reduced host cell invasiveness, given that the invasiveness of isolates without detectable Pls protein is similar to that of pls-negative MRSA isolates [18]. However, the exact molecular mechanism has remained unclear.

The aim of the present study was to determine the molecular mechanism of the reduced adherence and invasiveness caused by Pls. We tested the following hypotheses: (1) the effect of Pls could be an epiphenomenon that is dependent on SCCmec and the genetic background of S. aureus; (2) Pls could be secreted and then act by competing for potential substrate-binding sites or by binding to S. aureus adhesins or invasins; (3) Pls could interfere with host cell signaling; (4) the presence of the pls gene or the Pls protein could down-regulate adhesin or invasin gene expression; (5) Pls could interfere with adhesin or invasin surface expression; and (6) Pls could act by steric hindrance, limiting the access of adhesins or invasins because of its large size.

METHODS

Isolation and transformation of pPLS4. The plasmid pPLS4 was isolated from S. aureus strain 1061 Δpls (pPLS4) [17] by means of a Plasmid Mini Kit (Qiagen). pPLS4 was transformed into S. aureus strains Cowan I (methylcillin-susceptible Staphylococcus aureus [MSSA]) and Cowan I Δspa, 6850 (MSSA), and ST239-635/93 (MRSA and SCCmec type III). Parental and transformed strains were subjected to routine laboratory tests (Porex Staph-Plus [Bio-Rad] and API 20 Staph [bioMérieux]) for identification of staphylococci.

Cloning and expression of pPLS5 and pPLS6. The constructs were used full-length pls (pPLS4), pls-ΔLAPDTG (no sor- 
tase motif; pPLS5), and pls-ΔSD (no SD repeats; pPLS6). The polymerase chain reaction (PCR) product for pPLS5 was amplified from plasmid pPLS4 with forward primer Pls-5BF (5′-CGCGGATCCGCGTACCAATGAATATGGTTGTTACAAA-TAG-3′) and reverse primer Pls-5BR (5′-AACAGATAAAGG-3′). Two PCR products were separately prepared, ligated together after restriction, and then ligated into vector pCU1 [26], and transformed into Staphylococcus car- nosus TM300. A Pls-expressing clone was designated “TM300 (pPLS5).” For the construction of pPLS6, 2 sets of primers—Pls6BF (5′-CGCGGATCCGCGGTCATTCAATGAAATGATGTTGTTAC- AAATAG-3′) plus Pls6CR-1301 (5′-CGCATGATCTCATCTCTTAGGCAGTGGATTACGTCTCTC-3′) and reverse primer Pls6BR (5′-CGCGGATCCGCGTACCAATGAATATGGTTGTTACAAAATG-3′) plus Pls6CR-1301—were used. Two PCR products were separately prepared, ligated together after restriction, and then ligated into pCU1. A S. carno- sus TM300. Pls-expressing clone (1356 aa, 146.7 kDa, and identified with anti-Pls antiserum) was designated “TM300 (pPLS6).”

Purification of Pls, FnBPA, and ClfA and production of antiserum. Pls was purified from whole cell lysate of S. aureus 1061 by wheat germ agglutinin affinity chromatography, as de-
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Figure 3. Control for transcription of pls and fnbA.

Figure 4. Protein binding of Staphylococcus aureus strains Cowan I, 6850, and ST239-635/93 expressing (transformed with pPLS4) or not expressing (parental wild-type [WT]) Pls. A, Adherence to immobilized fibronectin (Fn). Polymethylmethacrylate coverslips were coated with either human serum albumin or Fn. A suspension of radiolabeled bacterial strains was added to the coverslips and incubated at 37°C with shaking. After 1 h, the bacterial suspension was removed, the coverslips were washed with phosphate-buffered saline (PBS), and adherent radioactivity was measured. B, Binding of soluble Fn. Microtiter wells were coated with S. aureus for 2 h at 37°C and subsequently blocked with 1% bovine serum albumin. Wells were washed after 1 h, and soluble biotinylated Fn (5 µg/mL) was added to adherent bacteria. Alkaline phosphatase–conjugated goat anti-rabbit antibody and alkaline phosphatase color substrate was used; then, avidine alkaline phosphatase and alkaline phosphatase color substrate was used. Optical density was determined at 405 nm. Results are means ± standard errors of the mean for at least 3 independent experiments performed in duplicate (A) or for 6 replicates (B), and adherence and Fn binding are expressed relative to those of WT Cowan I. Statistical significance was determined by the paired Student’s t test (2-tailed).
Figure 5. Invasion of 293 cells (A) and EA.hy 926 cells (B) by Staphylococcus aureus strains Cowan I, 6850, and ST239-635/93 expressing (transformed with pPLS4) or not expressing (parental wild-type [WT]) Pls. The total no. of internalized bacteria was measured by flow cytometry with fixed bacteria. Results are means ± standard errors of the mean for at least 3 independent experiments performed in duplicate, and invasiveness is expressed relative to that of WT Cowan I. Staphylococcus carnosus was used as a negative control. Statistical significance was determined by the paired Student’s \( t \) test (2-tailed).

For binding of soluble Fn, 5 \( \mu \)g/mL biotinylated Fn was added to each well after being washed with PBST, and the plate was incubated with shaking at 37°C for 1 h. The wells were then washed with PBST and incubated at 37°C for 1 h with 100 \( \mu \)L of a 1:10,000 dilution of anti-Fn antiserum (Dako). Wells were washed 4 times with PBST and filled with alkaline phosphatase color buffer, and 100 \( \mu \)L of phosphatase substrate (S0942; Sigma) was added. The plate was read within 20 min at 405 nm in a Versamax microplate reader (Molecular Devices).

For surface expression of FnBPs and Pls, wells were treated identically, except that anti-FnBPA or anti-Pls was added to wells after washing with PBST and incubation with shaking at 37°C for 1 h. Wells were washed, 100 \( \mu \)L of a 1:1500 dilution of alkaline phosphatase–conjugated goat anti-rabbit antibody was added to each well, and the plate was incubated at 37°C for 1 h. Cowan I \( \Delta spa \) was used to control for the effect of preimmune serum (figure 1).

Cell culture. The human endothelial cell line EA.hy 926 [30], provided by V. Gerke (Münster, Germany), was maintained in Dulbecco’s modified Eagle medium supplemented with 10% fetal calf serum (FCS) and antibiotics (50 IU/mL penicillin and 50 mg/mL streptomycin). We obtained 293 cells from the American Type Culture Collection (CRL-1573) and maintained them as described elsewhere [25, 31].

Flow cytometry invasion assay. Labeling of bacteria with fluorescein isothiocyanate and the invasion assay were performed as described elsewhere [31], with minor modifications [25]. Strain 6850 was used to control for the effect of preimmune serum (figure 2).

Real-time reverse-transcription PCR (rtRT-PCR). For RNA isolation, \( S. aureus \) was grown in brain-heart infusion medium to the desired growth phase. Bacteria were mechanically disrupted (FastPrep FP120; Qbiogene), and RNA was isolated using an RNasy Mini Kit (Qiagen). After treatment with RNase-free DNase I (Qiagen), total RNA samples were
amplified by means of the ABI Prism 7000 Sequence Detection System (Applied Biosystems), using SYBR Green PCR Master Mix (Applied Biosystems) and gyrB primers to check for the absence of genomic DNA. Previously transcribed complementary DNA (cDNA) served as a positive control. RNA was then reverse transcribed (High-Capacity cDNA Archive Kit; Applied Biosystems). For real-time amplification with specific primers, 100 ng of cDNA per reaction was used. mRNA expression of the different genes was normalized against constitutively expressed 16S ribosomal RNA gene as a reference gave similar results (figure 3). Primer sequences were as follows: for *spa*, *gyrB*, and *hla*, see Goerke et al. [33]; for *pls*, 5′-ACGTTCATTGATGGACGAC-3′ (forward) and 5′-TGTGTATTCTAGGGCTTC-3′ (reverse); for *fnbA*, 5′-AGAGGAATGGGAAGGATTCAC-3′ (forward) and 5′-GCGCGTGATGATTGTGTTT-3′ (reverse); and for *clfA*, 5′-CGCTATGCTATGGGACAA-3′ (forward) and 5′-AGCGCTATCAGGGTGTCA-3′ (reverse). Transcripts were quantified as the n-fold difference relative to the reference (2A_CT, where ΔCT represents the difference in the threshold cycle between the target and reference).

**Statistical analysis.** The paired Student’s t test (2-tailed) was used to determine the statistical significance of the results. Differences with *P*<.05 were considered significant.

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**RESULTS**

**Pls expression by transformants.** To detect any changes that resulted from the pPLS4 transformation, parental and transformed strains were subjected to routine laboratory tests. All tested strains were positive for rapid agglutination by the latex agglutination test for detection of Fg affinity (clumping factor), SpA, and capsular polysaccharides 5 and 8 of *S. aureus*. Biochemical identification kits did not show any differences between the parents and the respective transformed strains containing pPLS4. In sodium dodecyl sulfate polyacrylamide gel electrophoresis with Coomassie blue staining, Pls from all strains transformed with pPLS4 was seen as a major band of ∼230 kDa and a minor band of ∼175 kDa (data not shown). Pls was not observed in similarly prepared lysates from their respective *pls*-negative parental strains.

**Reduction in host protein binding and cellular invasiveness due to Pls expression.** In a solid-phase bacterial adhesion assay, strains expressing full-length Pls were ∼25% less adherent to Fn–coated polymethylmethacrylate coverslips than were their parental *pls*-negative strains (figure 4A). Similar results were obtained for immobilized Fg (data not shown). Binding of soluble Fn was reduced to a similar extent (figure 4B). We previously reported that *pls*-positive strains are less invasive than *pls*-negative strains for 293 cells [25]. Here, cellular invasion by Pls producers was reduced by up to 85%, compared with that of their respective parental strains without pPLS4. For both cell lines, the invasiveness of 6850 (pPLS4) and of ST239-635/93 (pPLS4) was strongly reduced (figure 5). Thus, Pls expression also reduced the invasiveness of MSSA strains independently of an SCCmec type I background.

**Effect of competition by purified Pls, recombinant FnBPA, and recombinant ClfA on invasiveness.** One potential mechanism of action of Pls could be competition for ligand-binding sites by secreted Pls. To investigate the effect of purified cell-surface proteins on cellular invasion, *S. aureus* Cowan I and purified/recombinant proteins (25 μg/mL) were added simultaneously to 293 cells. Pls and recombinant ClfA caused only a moderate reduction in cellular invasiveness (of 10% and 20%, respectively). By contrast, purified recombinant FnBPA caused an 85% reduction in cellular invasion (figure 6).

**Effect of anti-Pls and anti-FnBPA on invasiveness.** Pls could act by binding to an unknown host receptor, which in turn could down-modulate invasion. Thus, we tested whether anti-Pls could block the effect of Pls. High expression of SpA in the Cowan I strain interfered with blocking experiments in which antibodies were used. Thus, a SpA–deficient mutant (Cowan I ∆spa) was used for experiments with IgG fractions of anti-FnBP and anti-Pls antiserum. Anti-Pls had no effect on the invasiveness of all 3 wild-type strains tested. A moderate reduction in the anti-invasive effect of Pls expression could be observed (figure 7A). By contrast, anti-FnBP had a roughly
additive reducing effect on invasiveness (figure 7B). Similar results were obtained with anti-Pls F(ab')2 fragments for strains Cowan I Δspa (pPLS4), 6850 (pPLS4), and ST239-635/93 (pPLS4) (data not shown).

**Lack of mimicking of full-length Pls by Pls without the LPDTG motif or the SD repeat region.** To investigate the structural requirements for reduced invasiveness, we used 2 constructs. In plasmid pPLS5, the Pls LPDTG sortase motif is lacking. Thus, Pls cannot be anchored on the cell wall. In plasmid pPLS6, Pls is expressed without the SD repeat region. Thus, the protein is reduced in size, which potentially reduces the steric effect of this large protein. For 6850 (pPLS5), the invasiveness for 293 cells (figure 8A) and for EA.hy 926 cells (figure 8B) was only moderately reduced, much like with 6850 (pPLS4). This is in contrast to 6850 (pPLS4), which has an intact LPDTG motif. Cowan I (pPLS5) and Cowan I (pPLS6) showed a similar pattern, albeit with a lower reduction than for Cowan I (pPLS4) (figure 8). Thus, it appears that Pls must be anchored to the cell wall and be of full length for it to be functional.

**Up-regulation of fnbA and spa transcription but down-regulation of clfA and hla transcription due to Pls expression.** Transcription was monitored by rtRT-PCR for selected genes. As expected, pls transcription was observed in strains Cowan I (pPLS4), 6850 (pPLS4), and ST239-635/93 (pPLS4) but not in wild-type strains at 6 h (figure 9A). Surprisingly, fnbA transcription increased significantly in all 3 Pls-expressing isolates at 3.5 h (figure 9B). The effect of Pls expression on the transcription of fnbA, clfA, hla, and spa was further monitored in strain 6850. Transcription of fnbA was strongest in strain 6850.
Figure 8. Invasion of 293 cells (A) and EA.hy 926 cells (B) by *Staphylococcus aureus* strains Cowan I and 6850 transformed with different Pls constructs. Constructs used were full-length *pls* (pPLS4), *pls*-ΔPDTG (no sortase motif; pPLS5), and *pls*-ΔSD (no SD repeats; pPLS6). The total no. of internalized bacteria was measured by flow cytometry with fixed bacteria. Results are means ± standard errors of the mean for 3 independent experiments performed in duplicate, and invasiveness is expressed relative to that of wild-type (WT) Cowan I. *S. carnosus* was used as a negative control. Statistical significance was determined by the paired Student’s *t* test (2-tailed). NS, not significant.

(pPLS4), peaking at 3 h; *pls* transcription was low at 3 h and peaked at 5.5 h, considerably later than the peak of *fnbA* transcription (figure 9C and 9D). Transcription of *clfA* was low up to the 6-h time point and increased 2-fold at 10 h in wild-type 6850 but remained low in strain 6850 (pPLS4) (data not shown). Transcription of *spa* was very low in wild-type 6850 but increased at 3 h in strain 6850 (pPLS4) (data not shown). Transcription of *hla* was observed at 4 h and peaked at 5.5 h in wild-type 6850, whereas a low level *hla* signal was observed from 4 to 7 h in strain 6850 (pPLS4) (data not shown). Thus, *fnbA* transcription increased in Pls-expressing strains well before *pls* transcription peaked.

**Surface expression of Pls and FnBPs.** The signal for FnBP surface expression was more than doubled in Cowan I Δ*spa* (pPLS4), compared with that for the parental strain. Pls was detected only in Pls-expressing strains, and the results obtained for protein surface expression were in complete agreement with results of the rtRT-PCR assays. To overcome the high background observed with strain 6850 and IgG as anti-Pls or anti-FnBP (data not shown), biotin-labeled F(ab′)2 fragments of anti-Pls or anti-FnBP were used. The results showed a statistically significant increase in FnBP surface expression for 6850 (pPLS4), whereas Pls was not detected for the parental strain (figure 10A). FnBP and Pls surface expression were similar for a wild-type MRSA strain (SCCmec type I and Pls positive) (figure 10B). Thus, surface expression of FnBPs was increased, rather than decreased, for Pls-expressing strains, which was paralleled by marked surface expression of Pls. These results
Figure 9. Transcription of pls and fnbA. A and B, Real-time quantification of pls (A) and fnbA (B) gene transcription at 6 and 3.5 h, respectively, in Staphylococcus aureus strains Cowan I, 6850, and ST239-635/93 expressing (transformed with pPLS4) or not expressing (parental wild-type [WT]) Pls. C and D, Real-time quantification of pls (C) and fnbA (D) gene transcription in WT strain 6850 and 6850 (pPLS4) at the indicated time points. The calculated amount of individual messenger RNA is normalized against the amount of internal control (gyrB) transcription and is expressed as a fold increase. Data are means ± standard deviations for triplicate samples from each RNA extract. Statistical significance was determined by the paired Student’s t test (2-tailed).

DISCUSSION

We have previously demonstrated that the surface protein Pls of MRSA reduces adhesion to immobilized Fn, Fg, IgG, and laminin as well as invasion of host cells [17, 25]. The adhesion and invasion properties of MRSA strains devoid of Pls resemble those of MSSA strains, and reduced adhesiveness and invasiveness are associated with the pls gene found in SCCmec type I [16–18] and especially in type IV [18]. The importance of FnBPs for S. aureus is emphasized by the high prevalence of FnBPs among clinical isolates [34–36]. FnBP-mediated binding of bacteria to host cells certainly is the main route of efficient S. aureus host cell invasion. Consequently, a reduced Fn-binding capacity in strains that express Pls also leads to an impaired ability to invade host cells. Besides cellular invasiveness, Pls also reduces adherence of MRSA to several host proteins. Reduced cellular invasiveness appears to be the exception in clinical S. aureus isolates and to be mainly restricted to pls-positive MRSA [18, 25]. However, the mechanism has remained undetermined to date.

Here, we used pls-negative MSSA (strains 6850, Cowan I, and Cowan I Δspa) and MRSA (strain ST239-635/93; SCCmec type III) to express full-length Pls from plasmid pPLS4. Invasion of 293 and EA.hy 926 cells by the mutants was reduced by up to 85%, compared with that of the respective pls-negative parental strains. Pls-expressing strains were ∼25% less adherent to plastic surfaces coated with Fg and Fn and bound to soluble Fn comparably less. The rather moderate effect on protein binding is in line with previous observations that cellular invasion appears to be dependent on higher levels of FnPB surface expression [31]. The surface distribution of Pls on the wild-type or complemented strains is unknown. Variations in the spatial distribution of Pls may greatly affect the adhesive properties of a strain [17]. Pls probably has a general influence on adhesins that contain the LPXTG motif, because several distinct functions are affected. This hypothesis is supported by earlier experiments demonstrating that introduction of a mec element, which contains pls, caused MSSA strains to become methicillin

are in complete agreement with the results of the rtRT-PCR assays. Taken together, they demonstrate an increase in FnBP surface expression for Pls-expressing strains, despite their reduced invasiveness.
Figure 10. Pls and fibronectin-binding protein (FnBP) surface expression. A, Staphylococcus aureus strains Cowan I Δspa and 6850 expressing (transformed with pPLS4) or not expressing (parental wild-type [WT]) Pls. Microtiter wells were coated with Cowan I Δspa for 2 h at 37°C and subsequently blocked with 1% bovine serum albumin (BSA). Wells were washed after 1 h, and anti-Pls or anti-FnBP was added to adherent bacteria. Alkaline phosphatase–conjugated goat anti-rabbit antibody and alkaline phosphatase color substrate was used. Alternatively, microtiter wells were coated with strain 6850, and biotinylated anti-Pls or anti-FnBP F(ab′)₂ were used as antibodies. Then, avidine alkaline phosphatase and alkaline phosphatase color substrate was used. Binding to BSA was used as a control. Optical density (OD) was determined at 405 nm (OD 405). B. S. aureus WT strain 1061. Surface expression was determined for strain 1061 (staphylococcal cassette chromosome mec type I, pls positive, and Pls producing) and mutants as detailed for strain 6850 in panel A. Experiments were performed 3 times in triplicate, and results are presented as means ± standard deviations. Statistical significance was determined by the paired Student’s t test (2-tailed).

resistant and also weakly adhesive to immobilized Fn and Fg without a reduction in the expression of adhesins [37]. This would be compatible with a molecular mechanism by which Pls reduces adherence and invasion via decreased adhesin expression and competition for binding sites and/or anchoring sites as well as via steric hindrance.

Surprisingly, we found that Pls expression strongly up-regulated fnbA and spa transcription but down-regulated clfA and hla transcription. In previous reports, Pls-expressing strains have shown poor binding to Fg, which may, at least in part, be due to reduced expression of ClfA. Lack of clumping-factor activity has been reported for some MRSA strains [38]. In
contrast, SpA and FnBPA have been reported to be equally prevalent and expressed at similar levels in both pls-positive and pls-negative of MRSA. The few exceptions with regard to the expression of SpA correlate well with reduced adherence [25]. One potential mechanism for the transcriptional up- and down-regulation is that the multicopy plasmid used disturbs the balance of existing regulator elements. In this case, however, one would expect a parallel rather than an opposite effect for such genes as spa, fnbA, and clfA. In addition, the empty plasmid pCU1 had no effect (figures 3A, 3B, 11, and 12).

In line with previously published data, recombinant FnBPA caused a significant reduction (up to 90%) in cellular invasion for S. aureus. By contrast, purified Pls and recombinant ClfA reduced cellular invasion only marginally. A SpA mutant of Cowan I showed ~25% reduction in invasion with anti-FnBP. Anti-Pls had no major effect on down-modulated invasiveness by Pls. Taken together, these findings argue strongly against competition for substrate-binding sites. However, we could not differentiate between a mechanism where antibodies cross-link and thus bend Pls to the cell wall and a mechanism where antibodies interfere with additional host cell signaling events induced by Pls.

Pls contains 3 different types of repeat sequences. Extensive repeat regions are common in surface proteins of gram-positive bacteria, and some of them play a functional role in adherence [1, 6, 7, 39]. Pls can be regarded a member of the Sdr family. The repeat region R3 of Pls is mostly composed of the SD dipeptide, which is also found in the R regions of S. aureus and S. epidermidis surface proteins of the Clf-Sdr family [8, 9, 13, 19, 40]. The size of the ClfA R region varies among strains, and this region is probably for spanning the peptidoglycan layer and functions as an arm projecting the Fg-binding region outside the cell wall in a flexible manner [41]. The only common feature in the nonrepeat A region of the Sdr proteins is the consensus motif TYTFTDYVD [19, 40], which does not exist in the A region of Pls. Pls lacking the LPDTG motif (pPLS5) and the SD-repeat region (the “stem”) of Pls (cf. pPLS6) showed only a minor reduction in invasion Pls expression may ultimately be either an advantage or disadvantage for the fitness of Pls-expressing strains in vivo.

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