Staphylococcus epidermidis is the leading cause of device-related infections. These infections require an initial colonization step in which S. epidermidis adheres to the implanted material. This process is usually mediated by specific bacterial surface proteins and host factors coating the foreign device. Some of these surface proteins belong to the serine-aspartate repeat (Sdr) family, which includes adhesins from Staphylococcus aureus and S. epidermidis. Using a heterologous expression system in Lactococcus lactis to overproduce staphylococcal adherence redundancy we observed that one of these Sdr proteins, SdrF, mediates binding to type I collagen when present on the lactococcal cell surface. We used lactococcal recombinant strains, a protein–protein interaction assay and Western ligand blot analysis to demonstrate that this process occurs via the B domain of SdrF and both the α1 and α2 chains of type I collagen. It was also found that a single B domain repeat of S. epidermidis 9491 retains the capacity to bind to type I collagen. We demonstrated that the putative ligand binding N-terminal A domain does not bind to collagen which suggests that SdrF might be a multiligand adhesin. Antibodies directed against the B domain significantly reduce in vitro adherence of S. epidermidis to immobilized collagen.

Coagulase-negative staphylococci (CNS) are opportunistic pathogens that cause device-related infections such as those involving intravascular catheters and ventricular assist devices (VADs) (1–7). Staphylococcus epidermidis is the leading cause of these device-related infections accounting for 74–92% of the infections caused by CNS (8).

The pathogenesis of these infections is complex and involves a wide range of interactions between bacterial and host factors. However, this process usually requires an initial colonization step which allows bacteria to adhere to host tissues. In staphylococci these processes are commonly mediated by specific surface proteins called microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) which recognize and adhere to particular host factors (9–11). In the case of foreign body infections, the implanted surfaces quickly become coated with a wide range of extracellular matrix or plasma proteins such as fibrinogen (FnG), fibronectin (Fn), vitronectin (Vn), and collagen (Cn) (11–13).

Several S. epidermidis genes encoding proteins involved in adherence to host factors such as FnG, Fn, Vn, or Cn have been described (14–19). Some of these proteins have been shown to possess enzymatic properties in addition to their adhesive properties (16–18). One of these proteins, the FnG-binding protein serine-aspartate repeat protein G (SdrG), belongs to the serine-aspartate repeat (Sdr) subclass of MSCRAMMs which also includes SdrF and SdrH, whose specific ligands have not been identified (10). SdrF and SdrG possess features typical of other MSCRAMMs such as the cell sorting motif LPXTG, a hydrophobic region that spans the bacterial membrane and a C-terminal cluster of positively charged residues (10).

SdrF contains a 52-residue signal sequence followed by a putative ligand-binding domain, termed domain A and another region, domain B. Both of these domains can be subdivided into three and four subdomains respectively. Immediately following the B domain, a 558-residue region composed of repetitive serine-aspartate dipeptides, the SD-repeat region, is present, followed by the LPXTG cell wall-anchoring motif, membrane spanning region and positively charged residues (10). The SD-repeat region has been shown to be required for the proper display of the FnG-binding domain of the Staphylococcus aureus clumping factor A (ClfA) on the cell surface by spanning through the cell wall and has been suggested to have a similar function in other Sdr proteins (20).

We recently found that SdrF mediated S. epidermidis adherence to transcutaneous drivelines obtained from VADs explanted from patients with congestive heart failure. Histological examination of trichrome staining of driveline sections suggested that the main host factor coating the subcutaneous part of the driveline appeared to be Cn.4 Therefore we were inter-
SdrF from S. epidermidis Binds to Type I Collagen

TABLE 1
Plasmids created in this study

| Name       | Vector | Insert                                      | Oligonucleotides used | Features                      |
|------------|--------|---------------------------------------------|-----------------------|-------------------------------|
| pOri-SdrF  | pOri23 | Full-length sdrF                             | SDRF-5Bam, SDRF-3Pst  | L. lactis cell surface expression |
| pOri-SdrF8 | pOri23 | Full-length sdrF plus Ncol site              | SDRF-5Bam, SDRF-3Pst  | L. lactis cell surface expression |
| pOri-SdrFNA18 | pOri23 | sdrF lacking region A                        | SDRF-5Bam, F-SS + A1Nco | L. lactis cell surface expression |
| pOri-SdrFNAS6 | pOri23 | sdrF lacking region B                        | F-R5Nco, SDRF-3Pst    | L. lactis cell surface expression |
| pQE-rASdrF | pQE-30 | Region A of sdrF                             | F-His5A3m, F-His3Pst  | N-terminal His tag             |
| pQE-rBSdrF | pQE-30 | Region B of sdrF                             | F-His5B3m, F-His3Pst  | N-terminal His tag             |
| pQE-lukS  | pQE-30 | lukS-PV                                      | F-His5B3m, QEB1-3Pst  | N-terminal His tag             |
| pQE-rB1    | pQE-30 | B1 Repeat of sdrF                           | QEB2-3Pam, QEB2-3Pst  | N-terminal His tag             |
| pQE-rB2    | pQE-30 | B2 Repeat of sdrF                           | QEB3-5Bam, QEB3-3Pst  | N-terminal His tag             |
| pQE-rB3    | pQE-30 | B3 Repeat of sdrF                           | QEB4-5Bam, F-His3Pst  | N-terminal His tag             |
| pQE-rB4    | pQE-30 | B4 Repeat of sdrF                           | L. lactis cell surface expression |

Table 1: Plasmids created in this study

Plasmids created during this study are shown in Table 1. Oligonucleotides were designed to allow amplification by PCR of the appropriate DNA fragments generating specific restriction sites on both ends of the fragment (Table 2). The full-length sdrF gene as well as truncated versions lacking either the A or B domains were cloned into plasmid pOri23 (25) (kindly provided by P. Moreillon). These DNA fragments were subsequently digested with the suitable restriction endonucleases (New England Biolabs) and ligated to pOri23 previously digested with the same pair of restriction endonucleases. The full-length A and B regions of sdrF as well as the region encoding the mature LukS-PV polypeptide from S. aureus (26) were, in the same way, amplified by PCR, digested, and ligated to pQE-30 (Qiagen). L. lactis NZ9000 and S. aureus RN4220 were transformed as previously described (27, 28).

EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth Conditions—Escherichia coli XL1-Blue or XL10-Gold (Stratagene) were used as the first recipient strains in routine DNA cloning according to the manufacturer's instructions. S. aureus RN4220 (21) was used as the first Gram-positive host strain for receiving chimeric plasmids originated in E. coli before their transfer into their final host. S. epidermidis 9491 has been described previously (22). Lactococcus lactis NZ9000 (23) was used as the host strain for cell surface expression of recombinant S. epidermidis proteins. E. coli was grown at 37 °C in Luria Bertani broth (BD Biosciences). S. aureus was grown in Tryptic Soy broth (BD Biosciences) at 37 °C. S. epidermidis was grown at 37 °C in Tryptic Soy broth supplemented with 0.25% glucose. L. lactis was grown in M17 broth (BD Biosciences) supplemented with 0.5% glucose (GM17) at 30 °C. Mannitol Salt Agar was routinely used as solid media for S. epidermidis. Other solid media consisted of Tryptic Soy Broth supplemented with mutanolysin (100 units/ml) and lysozyme (100 µg/ml) for S. aureus and L. lactis strains; 5 g of type I Cn (Sigma) in 100 µl of PBS per well over-night at 4 °C. Wells were washed three times with PBS, blocked with 2% (w/v) nonfat dry milk (Bio-Rad) in PBS for 1 h, and washed five times with PBS.

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Expression and Purification of Histidine-tagged Recombinant Proteins—For the expression of recombinant LukS-PV (rLukS) and SdrF truncates, pQE-30 derived recombinant plasmids (Table 1) were cloned into E. coli XL1-Blue. Overnight cultures were inoculated into fresh medium and grown to an A500 of 0.3. Isopropyl β-D-thiogalactopyranoside was added to a concentration of 1 mM, and the culture was further grown for 4 h. Cells were harvested by centrifugation, resuspended in phosphate-buffered saline (PBS) supplemented with protease inhibitor (Roche Applied Science), imidazole (20 mM), and lysozyme (500 µg/ml) and incubated on ice for 30 min. Cells were lysed by sonication (Branson Ultrasonics), and cell debris was removed by centrifugation. Recombinant proteins expressed, containing an N-terminal polyhistidine fusion, were purified using HiTrap™ Chelating HP columns (GE Healthcare) according to the manufacturer's instructions and dialyzed extensively against PBS. Protein concentrations were determined using Bio-Rad Protein Assay (Bio-Rad).

Polyclonal Antibodies: Preparation and Purification—Polyclonal antibodies were produced at Covance Research Products Inc. by immunization of New Zealand White rabbits with purified recombinant A or B domains of SdrF (rASdrF or rBSdrF) as previously described (29).

Total IgGs from antisera directed against rASdrF (anti-rASdrF) and rBSdrF (anti-rBSdrF) were purified using Immunopure® (A) IgG purification kit (Pierce Biotechnology) according to the manufacturer’s instructions. Specific IgGs were further purified by affinity chromatography with MicroLink Protein Coupling kit (Pierce Biotechnology) according to the manufacturer’s instructions.

Solid Phase Assay to Measure Bacterial Adherence—Microtiter plates (MaxiSorp, Nalge Nunc International) were coated with 5 µg of type I Cn (Sigma) in 100 µl of PBS per well overnight at 4 °C. Wells were washed three times with PBS, blocked with 2% (w/v) nonfat dry milk (Bio-Rad) in PBS for 1 h, and washed five times with PBS.
cuitis cultures were centrifuged, bacterial cells were resuspended in PBS, adjusted to an $A_{600}$ of 1 or 0.5, respectively, and added to the microtiter wells. After 1 h at 37 °C, wells were extensively washed with PBS, and the adherent bacteria were collected using two sequential incubations with Trypsin/EDTA 1× (Invitrogen). Bacterial cell suspensions were serially diluted, plated onto the appropriate solid culture media and quantified after 24 h. Adherence to type I Cn was obtained for each sample by subtracting the number of adherent CFUs obtained from milk-coated wells to those lifted from Cn-coated wells. To measure the competition of Cn binding between *S. epidermidis* and purified proteins, blocked microtiter wells were preincubated with 80 μl of solutions at different protein concentration for 90 min at room temperature. *S. epidermidis* cell suspensions were adjusted to an $A_{600}$ of 5, 20 μl were added to each microtiter well, and incubation, washing, and collection of bacteria were carried out as mentioned above.

**Labeling of Recombinant Proteins**—Purified recombinant proteins were labeled with EZ-Link® NHS-LC-Biotin (Pierce) according to the manufacturer’s instructions.

**Solid Phase Assay of Protein-Protein Interactions**—The interactions between type I Cn and different truncated forms of SdrF were tested following a previously described protocol with minor modifications (18). Briefly, microtiter plates (MaxiSorp, Nalge Nunc International) were coated in a similar manner as described for the assay for bacterial adherence, blocked with 2% (w/v) bovine serum albumin (BSA) in PBS for 1 h, and biotinylated purified recombinant proteins (1 μg/ml unless otherwise stated) were added. Following 2 h of incubation at room temperature, wells were washed with PBS containing 0.05% (v/v) of Tween 20 (PBST) and subsequently incubated for 45 min with ImmunoPure® streptavidin, horseradish peroxidase-conjugated (Pierce) in blocking solution (0.1 μg/ml). Wells were then extensively washed with PBST and development was performed using 1-Step™ Ultra TMB-ELISA (Pierce) according to the manufacturer’s instructions. Absorbance at 450 nm ($A_{450}$) was measured using a Bio-Rad 680 Microplate Reader (Bio-Rad).

**SDS-PAGE and Western Blot**—SDS-PAGE and Western blots were performed by standard procedures (24) using Immobilon™-P transfer membrane (Millipore). Cell wall-associated proteins were extracted from *L. lactis* and *S. epidermidis* as previously described (25).

**Western Ligand Blot**—Samples were dissolved in Laemmli Sample Buffer (Bio-Rad), either boiled or incubated at 37 °C for 10 min, subjected to SDS-PAGE through a 10% polyacrylamide gel and transferred onto Immobilon™-P membrane (Millipore) using a Trans-blot® SD semi-dry Transfer Cell (Bio-Rad). Membrane was blocked with 5% (w/v) nonfat dry milk in PBST, washed three times with PBST, incubated for 2 h at room temperature with the appropriate purified recombinant protein in PBS (10 μg/ml) and washed three times with PBS. The membrane was then incubated overnight at 4 °C with the corresponding purified rabbit IgG, washed three times with PBST followed by incubation for 1 h with anti-rabbit IgG-peroxidase antibodies (Sigma) and three washes with PBST. Visualization was carried out with ECL™ Western blotting Detection reagents (GE Healthcare).

**RESULTS**

**Presence of SdrF on the Cell Surface of *L. lactis* Elicits Adherence to Type I Cn**—As mentioned above, our laboratory recently found that SdrF from *S. epidermidis* mediated adhesion to patient-explanted VAD drivelines when expressed and exported onto the lactococcal cell surface. Similarly it was observed that the major host component present on the surface of these implanted materials was Cn. Therefore, to determine whether cell surface-expressed SdrF can bind Cn a heterologous lactococcal expression system was first used to clone the full-length sdrF gene from *S. epidermidis* 9491 into the shuttle vector pOri23. Constitutive expression of sdrF and successful export of SdrF onto the lactococcal cell surface was then assessed by whole cell FITC labeling and flow cytometry analysis using both anti-rASdrF and anti-rBSdrF IgGs (Fig. 1A and supplemental Table S1). Adherence to immobilized solid-phase type I Cn was therefore tested. As previously reported, *S. epidermidis* 9491 bound type I Cn (Fig. 1B) (18) and *L. lactis* cells expressing SdrF on its surface were also able to bind type I Cn at a significantly higher level than *L. lactis* control cells containing only cloning vector pOri23 (Fig. 1B). *L. lactis* pOri-SdrF cells showed better binding capacity compared with *S. epidermidis* 9491, which might be explained by an increase in the presence of SdrF on the lactococcal cell surface as suggested by flow cytometry analysis. This, in turn, could be due to either increased protein expression or better presentation of SdrF antigens on the cell surface.

**Expression and Purification of rASdrF and rBSdrF from *S. epidermidis***—SdrF is composed of two putative ligand-binding regions (10, 22). To further characterize the Cn binding activity of SdrF, recombinant forms of these two regions, A and B (residues 53–677 and 678–1128, respectively) were cloned and expressed in *E. coli* with an N-terminal His tag (Fig. 2A). These polypeptides, with predicted molecular masses of 70 kDa for rASdrF and 51 kDa for rBSdrF, were subsequently purified by metal chelate affinity chromatography and dialyzed against PBS. Purity was confirmed by SDS-PAGE analysis (Fig. 2B). Both fusion proteins migrated with higher apparent molecular masses. Aberrant migration in SDS-PAGE has previously been observed in other *S. aureus* and *S. epidermidis* MSCRAMMs and might be caused by their hydrophilic nature (22, 31, 32).
These two purified polypeptides were used as antigens for the production of rabbit polyclonal antibodies.

The B Domain of SdrF Mediates the Adherence of L. lactis to Type I Cn—To further analyze the SdrF mechanism of binding to Cn we created a series of L. lactis strains harboring recombinant plasmids, which expressed different truncated forms of the SdrF polypeptide (Table 1). First, an NcoI restriction site was introduced between the A and B coding regions by DNA ligation of two PCR products comprising both ends of the full-length sdrF gene to pOri23 (Table 2) (Fig. 3A) thus obtaining plasmid pOri-SdrFN8. This plasmid was subsequently digested with either BamHI and NcoI, or NcoI and PstI, purified and ligated to the appropriate PCR product previously digested with the same pair of restriction enzymes (Fig. 3A).

The plasmids constructed in this manner, pOri-SdrFNA18 and pOri-SdrFN856, were then introduced in L. lactis NZ9000 for recombinant protein expression. Interestingly, we found that introducing the DNA fragment containing the mere sdrF signal sequence ligated to the B domain invariably yielded one or more mutations in this fragment (data not shown). Therefore, the DNA fragment containing the sdrF signal sequence had to be extended to include the first 22 codons of the A domain (Fig. 3A). Successful protein export and anchor to the lactococcal cell wall was demonstrated by flow cytometry analysis of whole cells using the total IgG fractions purified from polyclonal antiserum obtained by immunization of rabbits with purified rASdrF and rBSdrF (anti-rASdrF total IgGs and anti-rBSdrF total IgGs, respectively) (Fig. 3B). Flow cytometry showed an apparent increase in recombinant protein presence on the cell surface of A18 cells compared with SdrF cells (Fig. 3B). However, immunoblot analysis of cell wall-associated proteins showed similar levels of full-length SdrF and its truncated forms (data not shown). This observation may likely be due to a better accessibility to the B domain by anti-rBSdrF antibodies in cell surface-bound recombinant proteins lacking the N-terminal A domain. Therefore, relative levels of both SdrF-truncated forms appeared to be similar to that of the full-length SdrF polypeptide.

Once the presence of cell surface recombinant proteins had been demonstrated, Cn-binding levels were assessed for these L. lactis strains. It was thus observed that NZ9000 cells harboring pOri-SdrFNA18 (B domain) were able to bind to Cn-coated wells with a significantly higher affinity than control NZ9000 cells containing pOri23 (Fig. 3C). On the other hand, presence of the plasmid pOri-SdrF856 (A domain) in L. lactis cells did not significantly increase their ability to adhere to immobilized type I Cn (Fig. 3C). Cells expressing the full-length SdrF protein seemed to show a higher level of binding to type I Cn than those containing a truncated form lacking the A domain on their cell surface although the difference is not statistically significant (p = 0.12) (Fig. 3C). Taken together, these results suggest that the B domain is the main SdrF component involved in adher-
enge to type I Cn whereas the A domain does not mediate any adherence to Cn.

Recombinant Domain B, but Not Domain A, Binds to Type I Cn—The possibility that other region or regions in the mature truncated form of SdrF expressed by pOri-SdrFNA18 might also contribute to Cn binding led us to analyze this protein-protein interaction using a different approach. The collagen binding activities of the recombinant polypeptides encompassing rASdrF and rBSdrF domains were assessed using a solid-phase assay in which Cn-coated wells were incubated with either biotin-labeled SdrF putative ligand-binding domain and tested for Cn binding. Results showed that all four B repeats independently and purified the corresponding polyhistidine tagged peptides. These polypeptides were then biotinylated and tested for Cn binding. Results showed that all four B repeats elicited a significant level of binding to type I Cn (Fig. 5, supplemental Fig. S2). These data suggest that all four B repeats are able to independently mediate adherence to type I Cn.

Antibodies against the B Domain of SdrF Block the Adherence of rBSdrF to Both S. epidermidis and S. aureus leukocidin, was chosen as an irrelevant control protein for absence of adherence to type I Cn in the subsequent series of solid-phase assays. The DNA encoding the mature LukS-PV polypeptide was therefore cloned and expressed and its corresponding polypeptide purified and biotinylated (Tables 1 and 2). Significant differential biotinylation among some of the different preparations of purified rBSdrF and Reduce the Attachment of S. epidermidis to CnI

TABLE 2

Oligonucleotides used in this study

| Name       | Sequence (5′-3′)* | Endonuclease |
|------------|------------------|--------------|
| SDRF-5Bam  | AAAATTCCAACCTATGTCCTGCTAGGCTGCG   | BamHI        |
| SDRF-3Pst  | AAATTCCAACCTATGTCCTGCTAGGCTGCG   | NcoI         |
| SDRF-3Pst  | AAATATCCCCCTGTGCTGTTGAAG          | PstI         |
| SDRF-3Pst  | AAATATCCCCCTGTGCTGTTGAAG          | BstNI        |
| SDRF-5Bam  | AAAATTCCAACCTATGTCCTGCTAGGCTGCG   | BamHI        |
| SDRF-5Bam  | AAAATTCCAACCTATGTCCTGCTAGGCTGCG   | NcoI         |
| SDRF-5Bam  | AAAATTCCAACCTATGTCCTGCTAGGCTGCG   | PstI         |
| SDRF-5Bam  | AAAATTCCAACCTATGTCCTGCTAGGCTGCG   | BstNI        |

* Restriction sites are indicated in boldface type.

To further characterize this interaction between rBSdrF and solid-phase type I Cn different concentrations of the biotinylated protein were tested. The results show that rBSdrF adheres to type I Cn in a concentration-dependent, saturable manner (Fig. 4B). In this ligand-receptor interaction assay maximum binding occurs at about 20 ng/ml of rBSdrF.

Single B Repeats Can Mediate CnI Binding—The B domain of SdrF is composed of four repetitive amino acid sequences termed B repeats. These repeats are 119, 110, 111, and 111 residues long and have on average 55% identity at the amino acid level with each other. To further analyze the mechanism of binding of rBSdrF to type I Cn, we subsequently cloned all four B repeats independently and purified the corresponding polyhistidine tagged peptides. These polypeptides were then biotinylated and tested for Cn binding. Results showed that all four B repeats elicited a significant level of binding to type I Cn (Fig. 5, supplemental Fig. S2). These data suggest that all four B repeats are able to independently mediate adherence to type I Cn.

Antibodies against the B Domain of SdrF Block the Adherence of rBSdrF and Reduce the Attachment of S. epidermidis to CnI—The specific anti-rBSdrF IgG fraction from a previously obtained rabbit antiserum was purified. The effects of these specific IgGs on adherence to type I Cn of both rBSdrF and S. epidermidis strain 9491 were then examined. Purified rBSdrF or S. epidermidis 9491 cells were preincubated with increasing concentrations of anti-rBSdrF IgGs for 1 h before being added to Cn-coated microplate wells. The purified specific anti-rBSdrF antibodies reduced the attachment of rBSdrF in a dose-dependent manner (Fig. 6A). In the same way, S. epidermidis 9491 attachment to type I Cn was significantly reduced by anti-rBSdrF IgGs (Fig. 6B). In both cases purified preimmune IgGs had no perceptible effect (Fig. 6, A and B).

Purified rBSdrF Reduces the Adherence of S. epidermidis to CnI—Cn-coated microtiter wells were preincubated with increasing concentrations of either rLukS, rASdrF, or rBSdrF before incubation with S. epidermidis 9491 cells. Results showed that the presence of either rLukS or rASdrF did not produce any significant variation in the S. epidermidis attachment levels to type I Cn (Fig. 7). However, rBSdrF caused a significant reduction in the adherence level of S. epidermidis 9491 cells to type I Cn (Fig. 7).

rBSdrF Binds to Both a1- and a2-Chains of CnI—Type I Cn is typically composed of three polypeptide chains: two α1-chains and one α2-chain which, when analyzed by SDS-PAGE, result in a characteristic pattern of four bands distributed in two doublets with apparent molecular weights of 115 kDa and 130 kDa and another doublet at 215 kDa and 235 kDa (Fig. 8A) (33). We used a Western ligand blot in which type I Cn was subjected to SDS-PAGE, transferred to a polyvinylidene difluoride membrane and incubated with purified rASdrF or rBSdrF. After incubation of type I Cn with rASdrF and rBSdrF interactions were subsequently detected with the appropriate polyclonal
antibodies (anti-rASdrF or anti-rBSdrF) followed by anti-rabbit IgG-peroxidase. No interaction between rASdrF and immobilized type I Cn was detected (Fig. 8B), confirming our previous results which indicate that this putative ligand binding domain does not adhere to type I Cn. In contrast, incubation with purified rBSdrF produced a signal corresponding to the band formed by the two \( \alpha_1 \)-chains (Fig. 8B). This observation suggested that rBSdrF adhered to type I Cn via one or both \( \alpha_1 \)-chains. Interestingly, rBSdrF did not appear to bind to the blotted \( \alpha_2 \)-chain (Fig. 8B). A second band was produced by rBSdrF which probably corresponds to the one of the so-called \( \beta \)-dimers (33). To further investigate this observation we separately purified both types of \( \alpha \)-chains from type I Cn and subsequently tested the ability of rBSdrF to adhere to them using the previously mentioned solid phase assay of ligand-receptor interaction. Interestingly, we observed that rBSdrF adhered to both \( \alpha_1 \)- and \( \alpha_2 \)-coated microtiter wells (Fig. 9). However, it was also noticed that rBSdrF adheres with a significantly higher affinity to the \( \alpha_1 \) than to the \( \alpha_2 \)-chain (Fig. 9).

**DISCUSSION**

*S. epidermidis* is known to adhere to extracellular matrix components such as Vn, FnG, laminin, Cn, and Fn (16, 18, 34). This ability to adhere to a variety of host components is the major factor that makes *S. epidermidis* an increasingly important nosocomial pathogen and a frequent cause of indwelling device-related infections (35). Some of the proteins responsible for these adherent interactions have been identified although their relative contribution to this process remains uncertain (14, 15, 17, 18, 32, 36). Redundancy in surface proteins may confer an advantage for staphylococcal species in *in vivo* situations (9).

Several putative cell wall-anchored proteins of *S. epidermidis* have
we observed that these cells were able to bind to type I Cn, which suggested that SdrF was involved in this process.

SdrF belongs to the Sdr family of proteins and it has been postulated to possess three major regions, named A and B domains and SD region, as well as other features common to Sdr proteins (10). The S.D. region of the S. aureus clumping factor ClfA has been shown to act as a cell wall spanning domain that allows the more N-terminal part of the protein to be exposed on the cell surface (20). Using two recombinant L. lactis strains which produced and successfully exported onto their cell surface ligand binding domains A and B we found that the B domain was involved in binding to type I Cn. The A domain did not attach to the immobilized extracellular matrix factor. Interestingly, it has been recently hypothesized, based on sequence similarity analysis and secondary structure prediction, that the A domain of SdrF would be the most likely candidate to act as the ligand binding domain while the B domain would simply aid in the projection of the A region on the cell surface (10). Our results, however, strongly suggest that the B domain mediates Cn binding. In order to demonstrate that rBSdrF alone retains the ability to bind type I Cn we used purified biotinylated rBSdrF and rASdrF in an ELISA-like assay of protein-protein interaction. The class S component of the S. aureus Panton-Valentine leukocidin, LukS-PV (26), served as an irrelevant control example of a non-adherent protein. These tests confirmed previous results showing that rBSdrF adheres to immobilized type I Cn and that this adherence is not dependent on the presence of any other region of SdrF. These protein-
protein interaction tests also confirmed that rASdrF does not bind type I Cn. Finally, we also observed that this binding occurred in a dose-dependent manner.

In the same way, we tested the ability of purified recombinant proteins to block \textit{S. epidermidis} binding to type I Cn. The GehD lipase, the only other \textit{S. epidermidis} Cn-binding protein identified so far (18). These authors describe a half-maximum binding concentration of mature GehD of about 0.25 \( \mu \text{M} \) whereas in our case, this concentration appears to be somewhat lower (about 0.1 \( \mu \text{M} \)).
gesting a higher affinity of rBSdrF for type I Cn than that of GehD. Bowden et al. also postulated the existence of more than one cell surface adhesin mediating binding to Cn due to the partial rather than complete loss of adherence in a gehD mutant derived from *S. epidermidis* 9. Interestingly, we found that the *sdrF* gene in *S. epidermidis* 9, appears to have suffered a transitional mutation at position 71 (substitution of a cytosine for an adenine) which creates a stop codon (supplemental Fig. S3A).

We confirmed the lack of cell surface SdrF in anti-rBSdrF IgGs.

Alternatively, the possibility that GehD binding is either a more significant feature of this protein (18), or stoichiometrically less favorable than that of SdrF cannot be ruled out. This, together with the differences in the length of other *S. epidermidis* Cn-binding adhesins such as GehD. Alternatively, the possibility that GehD binding is either a more labile event, maybe due to the lack of cell wall anchoring typical of this protein (18), or stoichiometrically less favorable than that of SdrF cannot be ruled out. This, together with the different nature of the binding assay used by Bowden et al. (18) might explain the very dramatic reduction of binding achieved with anti-rBSdrF IgGs.

Type I Cn is a heterodimer composed of two α1 and one α2 chains which spontaneously form a triple helix scaffold under mild conditions (33). These chains are composed of tripeptide repeats and the two types differ mostly in the length of the polypeptide chains as well as in their carbohydrate substituions. We used Western ligand blot to confirm our previous findings and to visualize the adherence to the different types of α-chains and observed that rBSdrF appeared to bind only to α1-chains. To further investigate the difference in affinity shown by rBSdrF toward both types of Cn polypeptides we purified them independently and their ability to interact with rBSdrF was tested in a solid-phase binding assay. Interestingly it was observed that rBSdrF is able to bind to both chains in a solid phase assay, although its affinity for the α1-chain is significantly higher. Collagen single chains have a strong tendency to form trimers when in solution. Thus, the apparent inability of rBSdrF to adhere to membrane-bound α2-chain might be caused by their likely presence as monomers on the polyvinylidene difluoride membrane. In the case of α1-chains, this obstacle might be overcome by the increased affinity of rBSdrF. However, the possibility that rBSdrF can adhere to monomeric α1-chains or that these can fold into a triple helix once they have been transferred onto the membrane cannot be ruled out.

As mentioned above, rBSdrF of *S. epidermidis* 9491 is composed of four very similar subdomains termed B repeats. Results presented in this study constitute the first example of such a B repeat region from an Sdr protein serving as a ligand binding domain. These repeats show a high degree of identity (39–73%) with those repeats from the *S. aureus* proteins SdrC, SdrD, which have been shown to fold into a rod-like structure in a calcium-dependent manner and hypothesized to act as modulators for the projection of the A domain from the bacterial surface (37, 38), and SdrF (22). In addition they share a significant similarity with the B repeats from the *S. aureus* Cn-binding protein Cna which has been shown to fold in a zigzag pattern (39) and, in a similar manner, were also postulated to allow for the A domain to project away from the cell surface aiding in its correct presentation on the cell surface (10, 39). A “Collagen hug” molecular model for the binding of the A domain of Cna to Cn has been recently proposed (40). However, our results clearly indicated that rBSdrF is in fact able to act as a ligand binding domain and therefore, SdrF mechanism of binding to Cn must be different to that of Cna. To our knowledge only the repeat region of *S. aureus* FnBPA has been so far shown to possess a binding ability, in this case to Fn (41), even though a significant number of staphylococcal MSCRAMMs have been characterized to date. However, it must be noted that in this case, these D repeats from FnBPA have been shown to remain unfolded, adopting a structure only when they bind to the Fn molecule through a tandem β-zipper mechanism (42, 43).

We were interested in further investigating the mechanism of this interaction. Therefore a series of recombinant plasminids were cloned in *E. coli* and four different truncated versions of rBSdrF were purified. Each of these truncates contain one single B repeat from *S. epidermidis* 9491 fused to an N-terminal polyhistidine tag. Using our protein-protein interaction assay we observed that all of them appeared to retain their ability to attach to immobilized type I Cn. It seems that any of the four B repeats suffice to mediate by themselves appropriate levels of binding to type I Cn.

In summary this study demonstrates that SdrF, a member of the Ses family of proteins, is a collagen-binding protein. In contrast with earlier predictions, the B domain appears to be the critical domain involved in this process.

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