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Role of the *Staphylococcus epidermidis* Slime Layer in Experimental Tunnel Tract Infections

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An experimental animal model was used to assess the slime layer of *Staphylococcus epidermidis* as a pathogenic factor in tunnel tract infections. Mice were inoculated with high-slime-producing or non-slime-producing strains of *S. epidermidis*, either along the length of a subcutaneous catheter or in the area where a catheter had been placed and immediately removed (controls). Among the catheter-bearing mice, the phenotypically distinct staphylococci produced similar, high frequencies of abscess formation (72% [44 of 61] versus 81% [31 of 38]; $P = 0.29$). In controls, the non-slime-producing organisms were significantly more pathogenic (87% [40 of 46] versus 57% [25 of 44] abscess formation; $P = 0.001$). No consistent difference was detected between blood isolates obtained from patients with central venous catheter bacteremia and those from neonates with bacteremia in the absence of a prosthetic medical device. Quantitative culture of removed catheters showed greater adherence by the slime-producing isolates ($P = 0.014$). In this mouse model, slime production by *S. epidermidis* did not increase the risk of catheter tunnel tract infection, despite the greater catheter adherence of the slime-producing organisms. These findings suggest that traumatized tissue may be a sufficient condition for the development of *S. epidermidis* catheter-associated infections.

Staphylococcus epidermidis is an important infectious agent in a variety of clinical settings (28). It has been implicated as the principal etiologic agent in nosocomial bacteremias in low-birth-weight neonates (17, 26, 29, 33) and in cancer patients (23, 39, 40) and is a frequent cause of infections in patients with prosthetic medical devices, especially central venous catheters (CVCs) (18, 25, 32, 38). Locations of CVC infections include the catheter exit site (skin), the subcutaneous (tunnel) tract of the catheter, and the insertion site (32). Tunnel tract infections are particularly difficult to treat: only a third of patients respond to therapy without catheter removal (32).

The factors that permit *S. epidermidis*, a normal skin commensal, to become a nosocomial pathogen remain unclear. A slime substance, or exopolysaccharide, excreted by the organism in most CVC infections is thought by some investigators to shield CVC-attached *S. epidermidis* from phagocytosis (9). However, data on opsonophagocytosis in vitro are equivocal (21, 22, 36). Controversy also exists over the role of the slime substance in resistance to antibiotic therapy (12, 37) and in interference with the immune system (11, 14) and with coagulation (2, 11).

Most studies of *S. epidermidis* CVC infections have been conducted in vitro (10); however, Christensen et al. (6) developed a mouse model that permits assessment of *S. epidermidis* as a pathogen in tunnel tract infections. By using a modification of this system, we sought to determine the influence of the slime phenotype on abscess formation in the presence and absence of a subcutaneous catheter.

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MATERIALS AND METHODS

Bacterial strains and preparation. *S. epidermidis* isolates (A, B, and C) were blood isolates from three leukemia patients with CVCs, whereas isolates D, E, and F were obtained from three low-birth-weight neonates with bacteremia in the absence of CVCs (29). These latter three isolates were chosen because they allowed us to examine *S. epidermidis* infectivity without the bias of previous exposure to a CVC, which could be expected to select for organisms with increased virulence on catheter surfaces. The isolates were obtained from blood cultures and stored in buffered tryptic soy broth (TSB) with 10% glycerol at −70°C without serial passage. A commercially available identification system (American MicroScan; Baxter, West Sacramento, Calif.) was used for biotype analysis and to determine antibiogram susceptibility patterns, as defined by MICs.

Two bacterial preparations were used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Modified whole-cell lysates were prepared as previously described (29) and stored at −20°C. Cell wall extracts were prepared by a modification of the method of Cheung et al. (3), as described previously (30).

Slime layer quantitation. The spectrophotometric technique of Christensen et al. (7) was used to assess slime production. Overnight cultures in TSB were diluted 1:100 in fresh TSB, and aliquots of 200 μl were pipetted onto sterile microtiter tissue culture plates (Costar, Cambridge, Mass.). After overnight incubation at 37°C, the contents of each well were aspirated, and the well was washed four times with 200 μl of phosphate-buffered saline (PBS) (pH 7.2). Adherent organisms were fixed with Bouin's fixative for 5 min, rinsed, and stained with Hucker's crystal violet for 5 min. Excess stain was rinsed off with tap water, and the plates were allowed to dry. The optical density of stained adherent bacterial films at 570 nm (OD_{570}) was read with a microplate reader (Titertek Twin Reader; Flow Laboratories, McLean, Va.). Measurements were performed in quadruplicate on

three occasions and then averaged. The organisms were classified by their ODs: an OD of ≤0.120 indicated no slime production, an OD of 0.121 to 0.240 indicated moderate slime production, and an OD of >0.240 indicated high slime production.

Animal model. A modification of the mouse model of foreign body infection of Christensen et al. (6) was used. A 0.5-cm section of a 14-g Teflon catheter (Quick-Cath; Travenol Laboratories, Inc., Deerfield, Ill.) was inserted subcutaneously through a 0.5-cm incision in the interscapular area of 6- to 8-week-old CD-1 mice (Charles River Laboratories, Raleigh, N.C.). The wound was closed with a single stitch of 4.0 silk thread. Controls consisted of mice that lacked surgical incisions or that had catheter placement with removal before the incision was closed (sham operation). Additionally, a cohort of mice with catheters were injected with PBS (pH 7.2). The animals were allowed to rest for 3 days and then were examined for poor wound healing or infection. Animals with adequate healing were injected with 10^9 CFU of either slime-producing or non-slime-producing organisms, in 100 μl of PBS, along the length of the catheter or in the tract where the catheter had been placed. After 8 days, all animals were examined for the presence and location of abscesses. Catheters, if present, were removed, and quantitative cultures were performed by the method of Sherertz et al. (34). Briefly, catheters were placed in 1 ml of Columbia broth (Edge Biological, Inc., Memphis, Tenn.), sonicated in water for 1 min, and vortexed for 15 s. One hundred microliters of broth and a 1:100 dilution in PBS were plated onto Trypticase soy broth with 5% sheep blood (Edge Biologicals) by using a sterile glass hockey stick. The plates and the original broth were incubated for 48 h at 37°C, after which colonies were counted. Any *S. epidermidis* organisms isolated from cultures of abscesses or swabs of the surgical site were evaluated for biotype, antibiogram, and quantity of slime production. Abscess formation was defined as any grossly visible collection of purulent material, either on the catheter or in adjacent tissue. A single abscess was sufficient for an animal to be considered infected with *S. epidermidis*.

The minimal inoculum of slime-producing *S. epidermidis* isolate needed to cause abscesses in >90% of the mice with catheters was 10^9 CFU. This dosage did not provoke abscess formation in control mice that lacked incision sites and was used in all experiments with the animal model, whether the organisms were slime producers or non-slime producers.

Statistical methods. Chi-square analysis with Yates' correction was used to assess differences between proportions of animals with abscesses. Differences in catheter adherence (CFU per centimeter squared of catheter) between slime-producing and non-slime-producing isolates were evaluated by a Mann-Whitney U test.

RESULTS

The antibiotic susceptibilities, biotypes, and levels of slime production of the organisms are shown in Table 1. Four isolates (A, B, D, and E) were characterized as high-slime-producing isolates and two (C and F) were characterized as non-slime-producing isolates by the criteria of Christensen et al. (7). All six organisms had similar growth rates in vitro. The protein profiles of the isolates, obtained by SDS-PAGE analysis of whole-cell lysates and cell wall extracts, showed only minor differences; each isolate had major proteins with molecular masses of 36 to 37, 41, and 51 kDa (31).

TABLE 1. Characterization of *S. epidermidis* isolates[a]

Isolate	Biotype[b]	Antibiotic susceptibility[c]	Slime production (mean OD_{570} ± SD)
A	307164	RiF, V, Cp	1.084 ± 0.133
B	707124	Rif, Tet, V, E, Cp, Cd	0.360 ± 0.112
C	717164	V	0.036 ± 0.014
D	307064	Rif, Tet, V, E, Cd, TS	1.118 ± 0.137
E	703124	Rif, Tet, V, Cp, TS	0.620 ± 0.040
F	707164	Rif, V, Cp	0.043 ± 0.019

[a] Measurements were performed in quadruplicate on three occasions and then averaged. Isolates A, B, D, and E were classified as high slime producers (OD, >0.240) and isolates C and F were classified as non-slime producers (OD, ≤0.120) by the criteria of Christensen et al. (7).

[b] Determined by MicroScan analysis.

[c] Abbreviations: Rif, rifampin; Tet, tetracycline; V, vancomycin; E, erythromycin; Cd, clindamycin; Cp, ciprofloxacin; TS, trimethoprim-sulfamethoxazole.

Abscesses were observed in 72% (44 of 61) of the catheter-bearing mice inoculated with slime-producing organisms (A, B, D, and E) and in 81% (31 of 38) of those inoculated with the non-slime producers ($P = 0.29$; Fig. 1A). In mice with a sham operation, the slime-negative strains (C and F) were significantly more pathogenic than the four slime-positive strains (A, B, D, and E), causing abscesses in 87% (40 of 46) of the animals as compared with 57% (25 of 44) in the latter group ($P = 0.001$; Fig. 1B). No abscess was observed near the suture material. In a cross-comparison, slime-producing organisms (A, B, D, and E) induced abscesses more frequently in the presence of a catheter (72% [44 of 61]) than in the absence of a catheter (57% [25 of 44]), but this difference was not significant ($P = 0.10$). No abscesses were observed in control animals inoculated with PBS instead of *S. epidermidis* or in control animals inoculated with 10^9 CFU of any

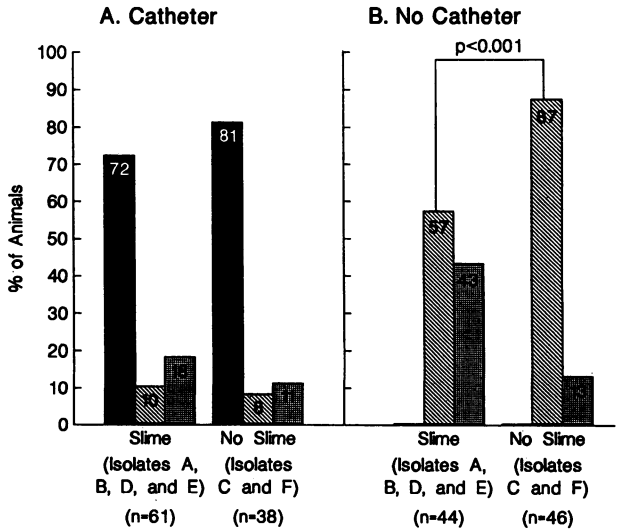

FIG. 1. Frequency of infection with slime-producing isolates A, B, D, and E and non-slime-producing isolates C and F of *S. epidermidis* in the presence (A) and absence (sham operation) (B) of subcutaneous catheters. Bars: ■, percent of animals with abscesses associated with catheters; ▧, percent of animals with abscesses not associated with catheters; ▦, percent of animals without abscesses. Statistical comparisons were performed by chi-square analysis with Yates' correction. Only significant differences are shown.

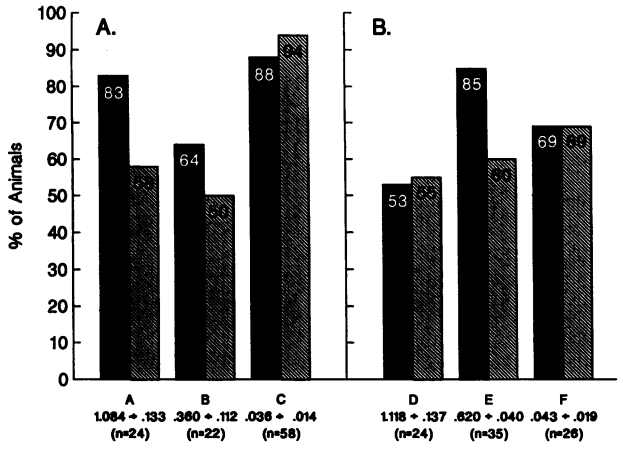

FIG. 2. Percentage of animals with abscesses from group inoculated with organisms from blood isolates of patients with CVC bacteremia (A) and from blood isolates from patients without CVC as depicted for slime quantitation (B). The strains used for inoculation (isolates A to F) are indicated below each pair of bars. Bars: ■, percent of animals with abscesses associated with catheters; ▨, percent of animals with abscesses not associated with catheters.

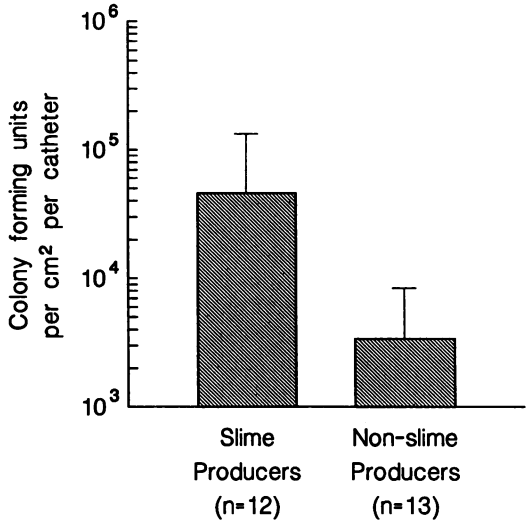

FIG. 3. Colonization of slime-producing and non-slime-producing organisms on subcutaneous catheters as measured in CFU per square centimeter of catheter. The slime-producing organisms were present in significantly higher densities ($P = 0.014$; Mann-Whitney U test).

of the six *S. epidermidis* isolates in the interscapular area without a surgical incision.

Comparison of abscess formation by organisms isolated from blood cultures of patients with CVC infections (isolates A, B, and C) with those organisms isolated from blood cultures of patients without CVCs (isolates D, E, and F) showed no consistent difference (Fig. 2). The non-slime-producing isolate C invoked abscess formation in the absence of a catheter (94% [31 of 33]) more frequently than did its comparative isolate (Fig. 2B, bars F; 69% [9 of 13]) obtained from a patient without a CVC ($P = 0.025$). It should be noted that more than 50% of the animals had abscess formation regardless of the phenotype of the organism or the presence or absence of a catheter.

Organisms that grew from swabs of injection sites occurred only in animals with visible abscesses. All isolates collected from swabs of the subcutaneous surgical sites or from quantitative catheter cultures were evaluated for biotype, antibiogram, and slime production. By use of MicroScan analysis, each isolate was identified as *S. epidermidis*, with an antibiogram and level of slime production identical to those of the parent strain. Selected isolates collected from infected animals were identical to the parent isolate as determined by SDS-PAGE analysis of whole-cell lysates and surface-exposed proteins (data not shown).

The ability of isolates to colonize catheters was assessed in catheters removed from 12 animals inoculated with slime-producing isolates (A, B, D, and E) and from 13 animals inoculated with the non-slime-producing isolates (C and F). As shown in Fig. 3, the slime-producing organisms grew to a significantly higher density than the non-slime-producing strains [mean ± standard deviation = $(4.6 \times 10^4) \pm (8.8 \times 10^4)$ versus $(3.4 \times 10^3) \pm (5.0 \times 10^3)$ CFU/cm^2 of catheter; $P = 0.014$)].

The presence of the catheter could have selected for strains with particular adherence characteristics; therefore, we compared slime-producing organisms isolated from the blood of patients with CVCs (isolates A and B) with slime-producing organisms isolated from the blood of patients without CVCs (isolates D and E) (Fig. 4). An organism's previous experience with a CVC did not result in selection of organisms which adhere to and colonize the catheter in greater numbers; indeed, the two slime-producing organisms isolated from patients without CVCs adhered and colonized in higher numbers ($P = 0.02$).

DISCUSSION

In this in vivo comparative study, slime-producing and non-slime-producing *S. epidermidis* isolates were associated with similar frequencies of abscess formation in mice with subcutaneous catheters in place. In sham-operated control animals, the non-slime-producing organisms were more pathogenic than the slime-producing strains, producing abscesses in significantly more animals (87% versus 57%). This

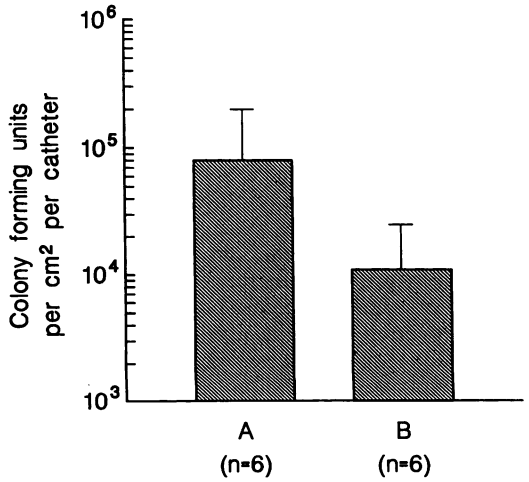

FIG. 4. Comparison of slime-producing isolates from blood isolates from patients without CVC for catheter colonization (A) and from blood isolates of patients with CVC bacteremia (B). The slime-producing organisms from patients without CVC adhered in higher densities ($P = 0.02$; Mann-Whitney U test).

suggests that slime may play a less critical role in the pathogenesis of coagulase-negative staphylococcal infections than was previously thought.

Bayston and Penny (1) were the first to observe that coagulase-negative staphylococci isolated from cerebrospinal fluid shunt infections produced a mucoid material that stained with alcian blue. Others have compared *S. epidermidis* isolated from skin or contaminated blood cultures with *S. epidermidis* from patients with septicemia or infected prosthetic devices and demonstrated the increased isolation of slime-producing strains from clinically significant infections (5, 8, 20). Christensen et al. (6) found that mice with catheters challenged with a slime-producing strain of *S. epidermidis* developed three times as many infections as mice inoculated with non-slime-producing organisms and that neither phenotype produced an infection when injected into sham-operated control animals. These same investigators later showed that there was considerable variation in slime production among adherent, slime-producing pathogenic strains of *S. epidermidis* and were able to correlate the results with the virulence of each strain, as determined by the 50% infective dose (4).

The reasons for the discrepancies between our findings and those of Christensen et al. (6) are not entirely clear. We explored the possibility that the origins of isolates selected certain phenotypic traits such as increased adherence to the catheter. No consistent difference in abscess formation between isolates obtained from patients with CVC bacteremia and those from patients with bacteremia but without CVCs could be demonstrated (Fig. 2). Moreover, isolates from patients without CVCs grew in higher densities on catheters than did isolates from patients with CVCs (Fig. 4). Thus, prior exposure of an isolate to a catheter does not appear to confer any enhanced virulence properties to the organism.

There is increasing evidence to suggest that bacterial adherence, persistence, multiplication, and infection may be distinct processes involving many factors besides slime production. Tojo et al. (35) recently isolated a polysaccharide adhesin that is required for adherence of some strains of *S. epidermidis* to silastic catheter tubing and may be produced by strains that do not elaborate slime. Thus, potentially pathogenic strains could produce adhesins but little or no slime before or early during growth on a subcutaneous catheter, a suggestion supported by the ultrastructural studies of Peters et al. (31). In addition, Pascual et al. (27) have shown that strain hydrophobicity is important in the initial adherence of coagulase-negative staphylococci to Teflon catheters. Our findings in an animal model confirm the suggestion that slime production may not be relevant to initial bacterial attachment to catheters. Moreover, the non-slime-producing isolate was more pathogenic in catheter-bearing animals than were the slime-producing strains, despite significantly greater catheter colonization by the latter. Clearly, factors other than slime-mediated colonization determine the infectivity of *S. epidermidis*.

The high frequency of abscess formation in sham-operated mice was unexpected. Both slime-producing and non-slime-producing organisms produced abscesses within the surgical field, consistent with recent studies showing staphylococcal adherence to host proteins, such as fibronectin and fibrinogen, that are involved in wound healing (15, 19, 24). This is supported by clinical studies of humans describing *S. epidermidis* infections of surgical wounds in the absence of a foreign body, such as mediastinitis following open heart surgery (13, 16).

The role of slime production in colonization of the catheter was investigated in vivo by using a modification of the quantitative technique of Sherertz et al. (34) for assessing the number of CFU of *S. epidermidis* per square centimeter of catheter. The slime-producing isolates produced significantly higher densities of organisms on the catheter (Fig. 3). These data support the ultrastructural scanning electron microscope observations of Peters et al. (31). These authors found no evidence of slime production during the initial adherence to catheters, but, with progressive colonization, slime was increasingly noted and there was a concomitant increase in the number of staphylococcal cells adhering to the catheter.

Our data support the hypothesis that factors other than a slime phenotype determine the pathogenicity of *S. epidermidis* in the presence of foreign bodies such as indwelling catheters. They further suggest that traumatized tunnel tract tissues can readily support *S. epidermidis* infections.

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