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Expression of Slime Interferes with In Vitro Detection of Host Protein Receptors of *Staphylococcus epidermidis*

LUCILLA BALDASSARRI,[1]* GIANFRANCO DONELLI,[1] ANTONELLA GELOSIA,[1] ANDREW W. SIMPSON,[2] AND GORDON D. CHRISTENSEN[2]

Laboratorio di Ultrastrutture, Istituto Superiore di Sanità, 00161 Rome, Italy,[1] and Research Service (151), Harry S. Truman Memorial Veterans' Hospital, Columbia, Missouri 65201[2]

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We hypothesized that slime may mask bacterial molecules important in the attachment of *Staphylococcus epidermidis* to inanimate surfaces. In support of this hypothesis, we found that slime-negative strains attached significantly better to fibrinogen or fibronectin than the parent strains and exhibited greater surface hydrophobicity. Comparable results were obtained with 53 clinical isolates.

Coagulase-negative staphylococci (CoagNS), particularly *Staphylococcus epidermidis*, are well-recognized pathogens of foreign body-associated infections (13). The pathogenesis of such infections involves an initial step of contact between the colonizing microorganism and the biomaterial, with subsequent colony formation. Thus, considerable attention has been given to the surface features of microbes that may play a role in the initiation of the colonization process.

Some investigations have indicated that matrix proteins absorbed on the implant surface can support the adhesion of *S. epidermidis* strains to various polymers (14, 19, 23), while others could not detect any increment in the rates of CoagNS adhesion to either silicone tubing (17) or Teflon catheters (18). Conflicting data also exist regarding the possible contribution of surface hydrophobicity to the ability of CoagNS to infect biomaterial implants (1, 11, 15, 22).

The present study was undertaken to test the hypothesis that slime, considered to be one of the most likely candidates for the CoagNS virulence factor (5), interferes with the in vitro function of certain surface components of *S. epidermidis* and impedes the evaluation of the roles they may play in the initial events of biomaterial-associated infections.

We have compared the surface characteristics of two *S. epidermidis* slime-positive strains and of slime-deficient or slime-negative mutants derived from these parental strains. The two slime-producing strains are *S. epidermidis* ATCC 35984 and *S. epidermidis* M187 (the latter kindly provided by G. Pier and E. Muller, Harvard University, Boston, Mass.). HAM892 (6) and D9 are slime-negative strains derived from ATCC 35984 by acryflavin mutagenesis. M187sn3 is a slime-negative mutant, also provided by G. Pier and E. Muller, derived from M187 by transposon mutagenesis (16). Fifty-three additional *S. epidermidis* strains isolated from catheter-associated infections were also studied.

All strains were grown overnight at 37°C in Trypticase soy broth under static conditions.

The binding ability of *S. epidermidis* to fibronectin, fibrinogen, and collagen type II and IV (all from Sigma; 50 μg/ml) was assessed by following the procedure described by Gatermann and Meyer (12). The adhesion of bacteria to immobilized proteins was relatively rapid, reaching 50% of the maximal levels within 30 min and completion within approximately 60 to 90 min (data not shown). There was essentially no change in the level of attachment after that point.

Significant differences were detected in the adhesion of strain M187 and its slime-negative derivative to fibronectin and fibrinogen (Fig. 1a) but not in their adhesion to collagen. A dramatic difference between *S. epidermidis* ATCC 35984 and its slime-negative derivatives with respect to adhesion to fibronectin and fibrinogen was also observed (Fig. 1b). Again, both HAM892 and D9 adhered at much higher levels than ATCC 35984 did, while no receptor to collagen seemed to be expressed. An up-to-sixfold increment in the adhesion to fibronectin of the mutants of ATCC 35984 was observed, as compared with a threefold increment for M187sn3. Increments in the adhesion to fibrinogen were about 25- to 30-fold in both cases.

To confirm the data obtained with the mutant-parent sets, 53 additional *S. epidermidis* strains of clinical origin were studied for their ability to bind to immobilized fibronectin, fibrinogen, and collagen. As shown in Fig. 2, the ability to bind to host proteins did not seem to be very common among the studied strains; most of them adhered equally well to protein- and to bovine serum albumin-coated wells, with a mean increase in the attachment to protein-coated substrates of lower than 10-fold. However, by grouping the strains as either slime negative or slime positive, it was found that fibronectin significantly promoted the adhesion of slime-negative strains (Fig. 2a) compared to slime-positive strains. A similar trend was observed for adhesion to fibrinogen (Fig. 2b); note that the two slime-positive strains most adherent to fibrinogen (177-fold and 111-fold increase) were weak slime producers ($0.120 < \text{optical density} < 0.240$). Adhesion to collagen was supported to a much lesser extent for all clinical isolates (Fig. 2c).

Results first suggesting the possible importance of adhesion to extracellular matrix proteins in the pathogenesis of CoagNS infections were those from Waldvogel and coworkers, who found up to a 100-fold mean increase of adhesion of *S. epidermidis* to polymethyl methacrylate treated with fibronectin (14, 24) and a 10-fold increment mediated by laminin or fibrinogen. There was, however, a wide range in the levels of adhesion exhibited by the individual strains in the CoagNS collection. Valentin-Weigand et al. (23) detected a 5- to 10-fold increase in the adhesion of staphylococci to fibronectin-coated polystyrene. Paulsson and coworkers (20) also reported on the ability of CoagNS to attach to extracellular matrix proteins but observed a wide variability in the adherence of 84 CoagNS clin-

* Corresponding author. Mailing address: Laboratorio di Ultrastrutture, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy. Phone: 39/6/4990.2092. Fax: 39/6/4938.7140.

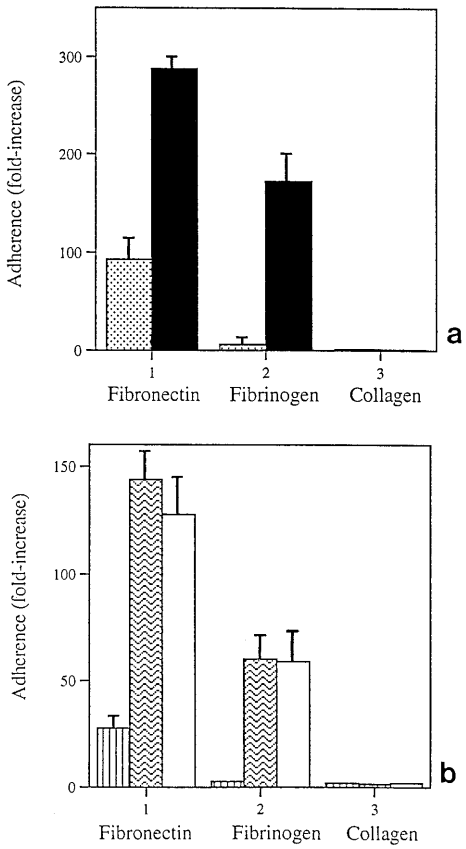

FIG. 1. (a) Adhesion of *S. epidermidis* M187 (▨) and the slime-deficient mutant M187sn3 (■) to immobilized fibronectin, fibrinogen, and collagen. (b) Adhesion to fibronectin-, fibrinogen-, and collagen-coated wells of *S. epidermidis* ATCC 35984 (▥) and its slime-negative derivatives HAM892 (▨) and D9 (□). Data are the average results (± SDs) of triplicate determinations of the increase of bacterial adherence to uncoated versus protein-coated surfaces.

ical isolates to protein-coated latex beads. In contrast, Muller and coworkers (17) did not find any enhancement of adhesion of CoagNS to silicone tubing by protein coating.

Our data indicate that the ability of a collection of clinical isolates of *S. epidermidis* to bind to immobilized host proteins is not a common feature and also shows great variability, with most of the strains being poorly promoted and only a minority being strongly promoted. However, the latter group included almost exclusively the slime-negative strains.

Surface hydrophobicity is another factor thought to be important in the close approach of organisms to surfaces. By measuring hydrophobicity according to the method of Rosenberg and coworkers (21), we observed that the fraction of *S. epidermidis* M187sn3 bound to *n*-hexadecane was much higher than that of the parent M187 cells (56 versus 30%), indicating that a generally higher hydrophobicity is associated with the reduced ability to produce slime. HAM892 and D9 made essentially no slime, and an even higher proportion of these cells bound to *n*-hexadecane (63 and 75%, respectively). These results were consistent throughout several repeated experiments performed over the course of the study. Hydrophobicity measurements made on parent strain ATCC 35984 were, however, not reproducible. The value we obtained, as a mean of more than ten experiments, was 48%, with a range of 25 to 67%. We believe that this nonreproducibility is primarily due to the difficulty in making a homogeneous suspension of these strongly slime-positive bacteria. A similar trend in hydrophobicity was observed for the clinical isolates, with the slime-negative strains being significantly more hydrophobic than the slime-positive ones (data not shown). However, again there were problems in preparing homogeneous suspensions of the very strong slime producers (optical density, >3.00) and thus obtaining reproducible measurements for them.

Slime on the surface of the organisms could contribute to hydrophilicity; since this polysaccharide material is loosely as-

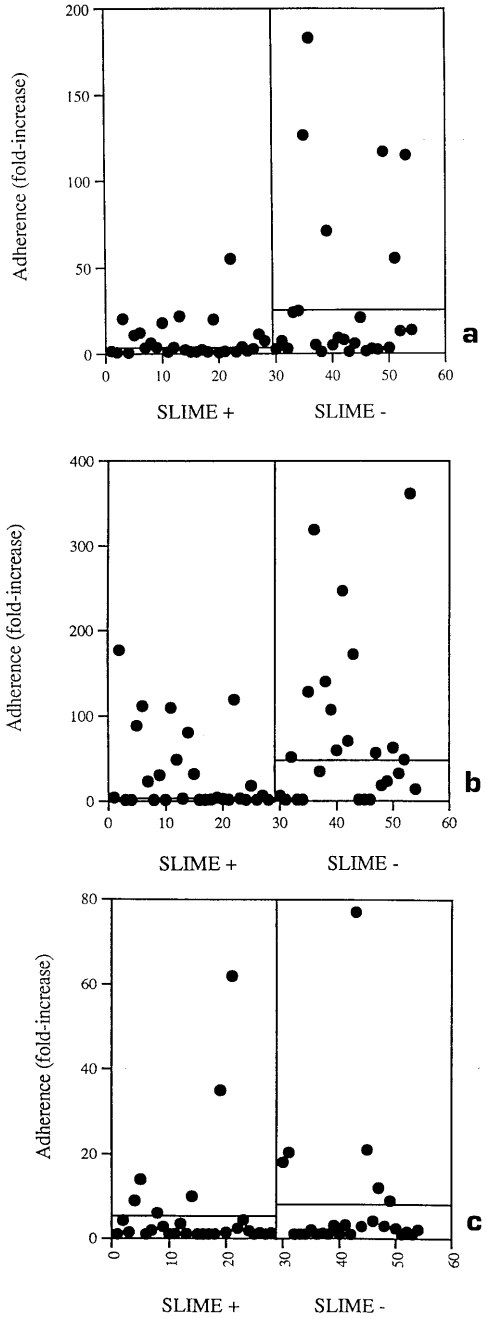

FIG. 2. Adhesion to fibronectin (a), fibrinogen (b), and collagen (c) of 53 *S. epidermidis* strains isolated from catheter-associated infections. The adhesion of slime-negative strains was significantly increased only by fibronectin and fibrinogen ($P < 0.02$). Dots indicate the average results of triplicate determinations of the increase of bacterial adherence to uncoated versus protein-coated surfaces; bars indicate the medians of the plotted values. Differences were analyzed for significance by the Wilcoxon test for related rankable scores.

TABLE 1. Slime production by *S. epidermidis* strains as measured by the plate test and image analysis of ultrathin sections

Strain	Slime production (OD_{570})[a]	Cell/slime ratio[b]
ATCC 35984	2.89 ± 0.02	2.81
D9	0.09 ± 0.01	0[c]
HAM892	0.08 ± 0.01	0
M187	2.77 ± 0.03	0.35
M187sn3	0.23 ± 0.07	0.15

[a] OD_{570}, optical density at 570 nm. Values are means ± SDs.
[b] Ratio of cell area to slime area as measured by morphometry.
[c] The value of 0 is arbitrary in that the production of extracellular material by these strains was almost undetectable.

sociated with the bacterial cells, one might speculate that it could also be lost in the suspending medium during experimental manipulations and thus could interfere to variable degrees with measurements of hydrophobicity for single cells. Unfortunately, difficulties in producing a uniform suspension of ATCC 35984, as well as of the strong slime producers from the clinical collection, make this analysis incomplete.

The higher hydrophobicities of the D9 and HAM892 strains compared to that of the slime-deficient M187sn3 strain and the more dramatic difference in the adhesion to fibronectin and fibrinogen of the isogenic set ATCC 35984, D9, and HAM892 compared to M187 and M187sn3 could be resolved by a more accurate measurement of slime production.

By following a modification (2) of the plate test described by Christensen and coworkers (8), strains ATCC 35984 and M187

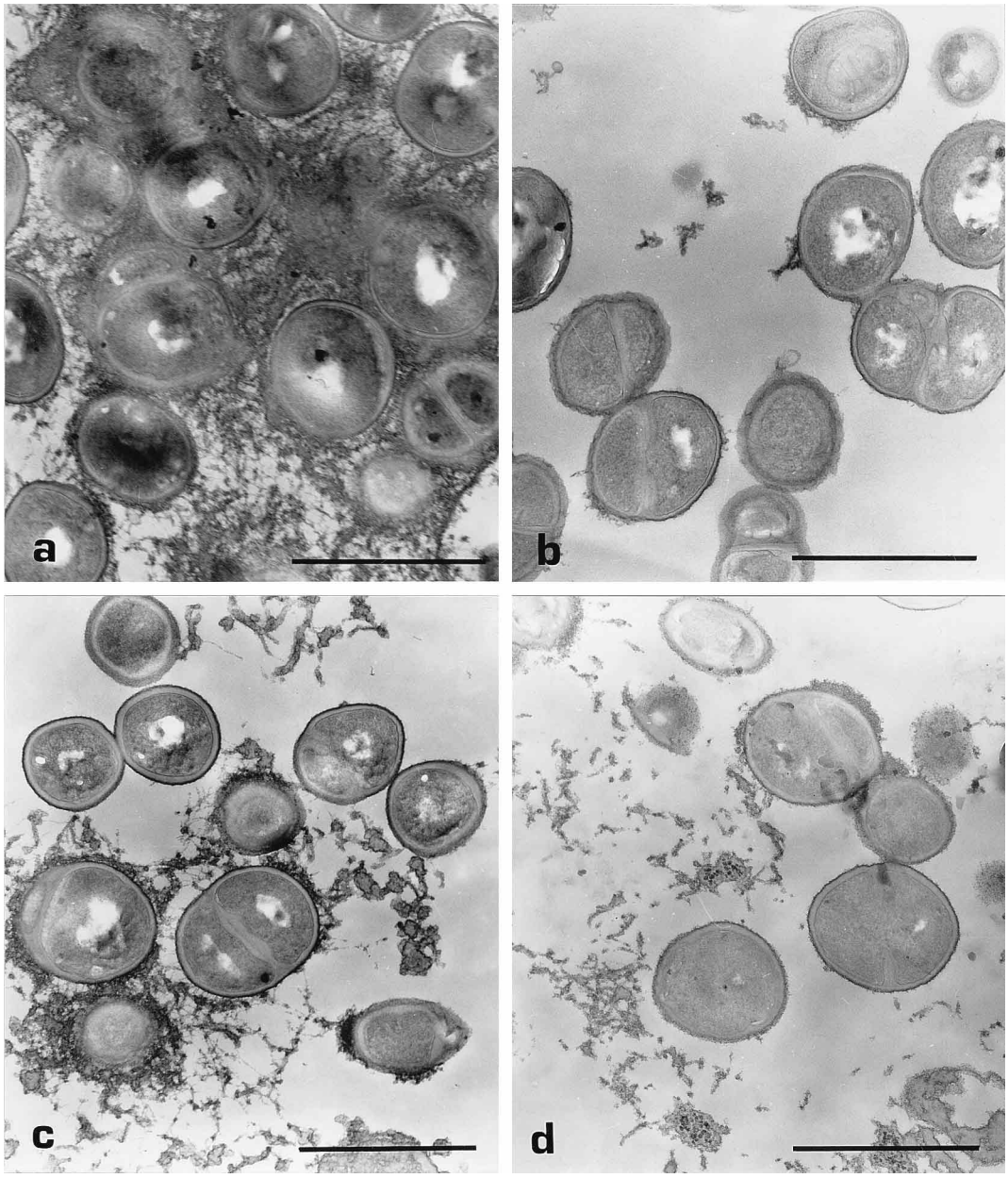

FIG. 3. Ultrathin sections of slime-negative and slime-positive strains. Note the abundant production of polysaccharide material by *S. epidermidis* ATCC 35984 (a) as compared either to that of the other slime-producing strain, M187 (c), or to that of slime-negative derivatives HAM892 (b) and M187sn3 (d). Bars, 1 μm.

appeared to produce comparable amounts of slime. However, we also performed a morphometric analysis on 20 randomly chosen fields on micrographs of strains ATCC 35984 and M187 taken at the same magnification. At least 100 cells were considered for each strain. Images were captured with a high-resolution camera, digitalized, and processed for computerized image analysis with an IBAS 2 system (Kontron-Zeiss). In the final calculations, the ratio of the cross-sectional area occupied by the bacterial cells to that occupied by slime was evaluated. Image analysis showed that ATCC 35984 produced significantly greater amounts of slime than M187. *S. epidermidis* ATCC 35984 had a cell area of 4,963 ± 1,846 (mean ± standard deviation [SD]; values are expressed in the arbitrary units assigned by the image analyzer) and a slime area of 14,612 ± 6,660, giving a slime area/cell area ratio of 2.81. For comparable values of cell area, *S. epidermidis* M187 showed a slime area of only 2,340 ± 1,750, and therefore a slime area/cell area ratio of 0.35. These differences were statistically significant ($P < 0.001$) and indicated that the relatively small differences between these strains as determined by the plate test for slime production were somewhat misleading. The analysis also confirmed that M187sn3 continues to produce a certain amount of ruthenium red-positive extracellular material (slime area/cell area ratio = 0.15), especially compared to HAM892 and D9, which produced virtually no extracellular material (slime area/cell area ratio = 0.0) (Table 1).

To ensure that the preparation procedure for electron microscopy did not affect slime preservation we tried to stabilize slime with Alcian blue, by pretreatment with immune antiserum or lectins (data not shown), and by using the procedure of Fassel and coworkers (10), which allowed the best preservation of slime (Fig. 3).

The possibility that the production of slime may mask the presence of other surface structures that are important adhesion factors has often been suggested but, to our knowledge, never demonstrated. Only recently have investigators begun to give formal attention to this subject. Two of the authors (7) recently observed that antibody titers against slime-positive *S. epidermidis* ATCC 35984 in patient sera were always lower than titers against slime-negative mutant HAM892 derived from it. Also, Yu et al. (25) reported that *S. epidermidis* strains grown in the presence of glucose to enhance slime production adhered less avidly to heparinized polymer surfaces. Besides the presence of glucose, slime production is known to be strongly affected by growth conditions (3, 9) and phenotypic variation (4). Our data also suggest that the measurement of slime production by the plate test may be somewhat misleading as to the real slime-producing ability.

In this study, the production of slime seemed to be the discriminative factor between strongly adherent and weakly adherent strains and between hydrophobic and hydrophilic strains, both for the isogenic sets and for the clinical isolates. Due to the differences between the parent strains, to the incomparable mutagenesis procedures followed to create the derivative strains, and to the still relatively uncharacterized nature of the mutation involved, it may be hazardous to draw a definitive conclusion; however, the fact that some of the slime producers showed an enhanced adhesion to fibronectin and fibrinogen after mild sonication (data not shown) further supports our interpretation, i.e., that slime may be responsible for masking the ability of the parent strains to bind to immobilized host proteins.

In this regard, the observations reported call attention to the presence of extracellular matrix-reactive adhesins and/or hydrophobic molecules on the surface of *S. epidermidis* that appear to be masked by the presence of the slime. Slime production should thus be taken into account, not only as an important virulence factor in its own right, but also as a factor that may interfere with our ability to detect the activities of other surface molecules.

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