INFECTION AND IMMUNITY, Feb. 1997, p. 519–524 Vol. 65, No. 2
0019-9567/97/$04.00+0

A 140-Kilodalton Extracellular Protein Is Essential for the Accumulation of *Staphylococcus epidermidis* Strains on Surfaces

MUZAFFAR HUSSAIN,[1] MATHIAS HERRMANN,[1]* CHRISTOF VON EIFF,[1] FRANÇOISE PERDREAU-REMINGTON,[2] AND GEORG PETERS[1]

Institute of Medical Microbiology, University Hospital, Muenster,[1] and Institute of Medical Microbiology and Hygiene, University Hospital, Cologne,[2] Germany

Received 12 September 1996/Returned for modification 28 October 1996/Accepted 18 November 1996

Two distinct pathogenic mechanisms, adhesion to polymer surfaces and subsequent accumulation of sessile bacterial cells, are considered important pathogenic steps in foreign body infections caused by *Staphylococcus epidermidis*. By using mitomycin mutagenesis, we have recently generated a mutant, strain M7, from *S. epidermidis* RP62A which is unaffected in adhesion but deficient in accumulation on glass or polystyrene surfaces and lacks a 115-kDa extracellular protein (designated the 140-kDa antigen; F. Schumacher-Perdreau, C. Heilmann, G. Peters, F. Götz, and G. Pulverer, FEMS Microbiol. Lett. 117:71–78, 1994). To evaluate the role of this protein in accumulation, we harvested extracellular proteins from *S. epidermidis* RP62A grown on dialysis membranes placed over chemically defined medium, purified the protein by using ion-exchange chromatography, determined its N-terminal amino acid sequence, and raised antiserum in rabbits. The antibody recognized only a single band in a Western immunoblot of the crude extracellular extract. With the microtiter biofilm test, antiserum at a dilution of ≤1:1,000 blocked accumulation of RP62A up to 98% whereas preimmune serum did not. The 140-kDa antigen was found only in extracellular products from bacteria grown under sessile conditions. Of 58 coagulase-negative clinical isolates, 32 strains were 140-kDa antigen positive and produced significantly larger amounts of biofilm than the 26 strains that were 140-kDa antigen negative. The 140-kDa protein appears to be biochemically and functionally unrelated to any previously described factors associated with biofilm formation. Thus, the 140-kDa antigen, referred to as accumulation-associated protein, may be a factor essential in *S. epidermidis* accumulation and, due to its immunogenicity, may allow the development of novel immunotherapeutic strategies for prevention of foreign body infection.

Coagulase-negative staphylococci (CoNS) have emerged as the most frequently isolated pathogens in nosocomial sepsis (28) and cause more infections of medical devices than any other group of microorganisms (for a review, see reference 4). In contrast to *Staphylococcus aureus*, which is thought to adhere to polymeric surfaces in vivo mainly through interaction of staphylococcal surface receptors with surface-adsorbed host factors (42), CoNS may colonize unadsorbed plastic material, e.g., a catheter at its insertion site or a prosthesis during the implantation procedure, by forming a biofilm consisting of cells and extracellular material (6, 9, 38, 43, 44). While biofilm formation is thought to be of importance for CoNS virulence (6, 7, 10–12, 26, 56), the mechanisms involved in biofilm formation are not fully understood. Two distinct pathogenic mechanisms have been suggested to contribute to colonization and biofilm formation (5). While the rapid initial adhesion of bacterial cells to a polymer surface is thought to be mediated primarily by bacterial adhesins of a proteinaceous and/or carbohydrate nature (5, 19, 53), accumulation of multilayered cell clusters embedded within an exopolymer substance is a process which occurs over a period of hours and has been associated with the presence of an *S. epidermidis* polysaccharide antigen (37).

We generated a mutant, strain M7, of *S. epidermidis* RP62A (ATCC 35984) that is indistinguishable in all of the phenotypic and genetic characteristics tested, except that it fails to accumulate when attached to plastic or glass (46). This mutant was shown to lack a 115-kDa protein (later shown to be a 140-kDa protein). The purpose of the present study was to determine whether the presence of this protein in clinical isolates is associated with biofilm formation and whether an antibody to this protein can inhibit biofilm formation.

* Corresponding author. Mailing address: Institute of Medical Microbiology, University of Muenster Hospital and Clinics, Domagkstr. 10, 48129 Muenster, Germany. Phone: IAC-49-251-835-5360. Fax: IAC-49-251-835-5350. E-mail: herrmam@uni-muenster.de.

MATERIALS AND METHODS

A bacterial suspension containing 10^6 CFU of either *S. epidermidis* RP62A or M7 per ml in 0.9% NaCl was prepared from a Muller-Hinton agar plate incubated overnight, and 2 ml was inoculated onto a washed sterile dialysis membrane (12 by 8.6 cm; 14-kDa cutoff) that had been laid over chemically defined medium (CDM) agar (54) in a petri dish (12 by 12 cm). Particular care was taken to avoid any contact between the bacterial solution and the agar surface. After incubation (18 h, 35°C), the extracellular protein (ECP)-bacteria mixture was collected in a sterile flask and the dialysis membrane was washed once with 0.3 ml of normal saline. This was then sonicated twice (15 s, 35 W) (Branson Sonifier 25; Branson Inc., Danbury, Conn.) and centrifuged at 12,500 × *g* and 4°C for 15 min to remove bacteria. To 30 ml of supernatant, 120 ml of Tris HCl (25 mM, pH 7.8) was added; the mixture was then concentrated to 10 ml in an ultrafiltration cell (Filtron membrane cells, Nova series; Filtron GmbH, Karlstein, Germany) with a molecular mass cutoff of 10 kDa. The concentrated ECP was stored at −20°C.

ECP was fractionated with an anion-exchange Bio-Scale Q2 column (Bio-Rad Laboratories GmbH, Munich, Germany) connected to the Biologic System (Bio-Rad) with a linear gradient of NaCl (0 to 1 M) in Tris-HCl (25 mM, pH 7.8). Concentrated ECP and fractions were analyzed by Coomassie blue-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 7.5 or 12% separating gel and 4% stacking gel). To avoid proteolytic degradation of purified proteins, 1 mM (final concentration) phenylmethylsulfonyl fluoride (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany), 1 mM EDTA (Sigma), and 2 mM *N*-ethylmaleimide (Sigma) were added to all of the buffers used to harvest, concentrate, and chromatographically purify ECP. Amino-terminal amino acid sequencing was performed with approximately 100 pmol of purified protein on

an automated ABI amino terminus sequencer (Applied Biosystems Inc., Weiterstadt, Germany), and searches for sequence homology were performed with PC/Gene (IntelliGenetics Inc., Mountain View, Calif.). The fractions containing the purified 140-kDa antigen were further examined for nucleic acid with ethidium bromide-stained agarose gels, for carbohydrate and phosphate contamination by acid hydrolysis and thin-layer chromatography (22, 23), and for phosphorus (1), hexosamines (32), and neutral sugars (14) by standard assays.

For immunoblotting, polyclonal antibodies against the purified protein were raised in two rabbits by standard procedures (18). After collection of preimmune sera, each rabbit was immunized subcutaneously with 50 μg of antigen in complete Freund's adjuvant (Sigma). Second and third injections of antigen in incomplete Freund's adjuvant were given subcutaneously 2 and 4 weeks later, respectively. Blood was obtained 2 weeks after the last antigen injection. Naturally occurring antistaphylococcal antibodies were complexed by mixing serum with 10 volumes of ECP from mutant strain M7, which does not produce the 140-kDa protein, and immunocomplexes were partially removed by centrifugation (14,000 × *g*, 5 min). ECPs were separated on a 7.5% separating gel, transferred (FastBlot; Biometra, Göttingen, Germany) onto a nitrocellulose membrane (Schleicher & Schüll, Dassel, Germany), and probed with antisera. Bound rabbit immunoglobulin G were detected with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulins (Dako Diagnostika, Hamburg, Germany) and the alkaline phosphatase reaction (Bio-Rad) in accordance with the instructions of supplier.

To evaluate inhibition of accumulation by the antisera raised, the microtiter plate method for determination of adherent bacteria (8) was used with slight modifications. *S. epidermidis* RP62A grown on blood agar plates was added to liquid CDM containing dilutions of antiserum raised against the purified 140-kDa protein. Triplicate samples (each containing 0.2 ml) were added to individual wells in a polystyrene 96-well flat-bottom microtitration plate (Nunc GmbH, Wiesbaden, Germany). Microtiter plates were incubated initially at 4°C for 2 h and then at 37°C for 18 h. Adherent biofilm was stained with safranin dye for 1 min and washed with distilled water, and the optical density at 492 nm (OD_{492}) was determined. Percent inhibition of accumulation was calculated by using the following formula: $[1 - (OD_{492[\text{with antiserum}]}/OD_{492[\text{without antiserum}]})] \times 100$. The Mann-Whitney rank sum test was employed to compare OD_{492} values of groups of strains, and the Fisher exact test was used for comparison of biofilm formation of isolates on glass by using a tube test (6). The tube test (6) was also used to observe the effect of antisera raised against the purified protein on accumulation.

RESULTS

As reported earlier (46), the parent strain produces 29- and 115-kDa ECPs which are not produced by mutant M7. The approximate molecular masses of 29 and 115 kDa were estimated by SDS-PAGE with a 12% separating gel. We confirmed these findings but found that the 115-kDa protein showed different mobilities in different SDS-PAGE systems, with apparent molecular sizes varying between 120 and 180 kDa. Computer-assisted two-dimensional gel electrophoresis of ECPs obtained from strain RP62A and mutant strain M7 confirmed the absence of proteins with apparent sizes of 140 and 20 kDa in ECP from the mutant and the presence of these proteins in ECP from the parent strain (data not shown). Accordingly, the 120- to 180-kDa protein was designated the 140-kDa protein. This protein was present only in ECP obtained from RP62A grown under sessile, but not under planctonic, growth conditions, and it was not detected in ECP from mutant strain M7 by SDS-PAGE performed in one (Fig. 1, lane A) or two dimensions (data not shown).

The 140-kDa protein was purified by medium-pressure anion-exchange chromatography. Fractions eluted at 0.1 M NaCl in Tris-HCl buffer (25 mM) contained different quantities of the 140-kDa protein (99.9% pure as assessed by both Coomassie blue- and silver-stained SDS-PAGE) (Fig. 2, lanes A and B). Fractions were negative for nucleic acids, carbohydrates, and phosphorus. Antiserum at dilutions as high as 1:50,000 revealed a positive 140-kDa protein signal in crude ECP (Fig. 3, lane B) and after purification (Fig. 3, lane C) from strain RP62A in Western blot assays, whereas a 1:10,000 antibody dilution did not reveal a 140-kDa protein in ECP from mutant strain M7 (Fig. 3, lane A). The purified 140-kDa protein was subjected to N-terminal amino acid sequence analysis, and the following sequence was obtained:

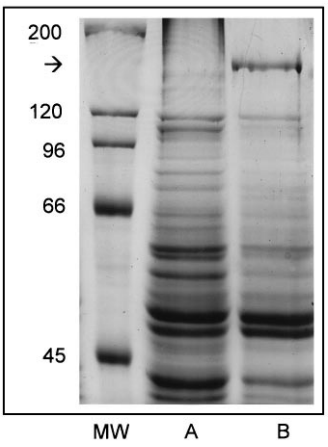

FIG. 1. Coomassie-blue stained 7.5% polyacrylamide gel of ECPs. Bacteria were grown overnight on dialysis membranes on CDM agar, the ECP-bacterium mixture was collected and centrifuged, and the cell-free supernatant was concentrated. Lanes: A, ECP of *S. epidermidis* M7; B, ECP of *S. epidermidis* RP62A; MW, molecular mass markers, whose sizes are indicated in kilodaltons on the left. Arrow, 140-kDa protein.

$$\overset{V}{A}TQ\overset{S}{M}T(A)NVS(G)\overset{T}{K}QTYQDPTYV\overset{Q}{D}(P)(K)$$

The nine-amino-acid unambiguous sequence was evaluated for homology with the SwissProt database, and no deposited protein sequence showed a high level of homology (≥67%).

The ECP was prepared from 46 strains of *S. epidermidis* and from 12 other CoNS strains obtained from patients with intravenous catheter-associated bacteremia or endocarditis. Only one isolate per patient was tested in this study. Proteins in crude extract were separated by SDS-PAGE and transferred to nitrocellulose membranes. Antigens blotted onto nitrocellulose membranes were probed with antisera to the 140-kDa protein raised in rabbits. Most of the 140-kDa antigen-positive strains formed a biofilm on microtiter plates (median OD_{492}, 0.35 [range, 0.0 to 2.0]), whereas most of the strains negative

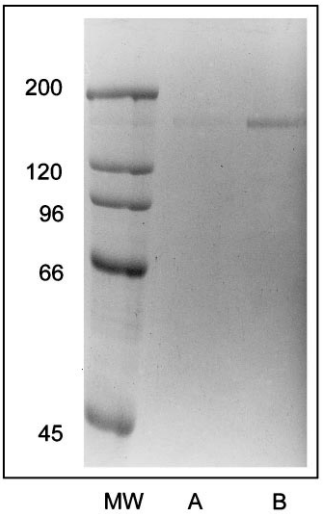

FIG. 2. Purified 140-kDa protein from *S. epidermidis* RP62A. ECP was prepared as described in the legend to Fig. 1 and fractionated on a BioScale Q2 column with a linear gradient of 0 to 1 M NaCl. At 0.1 M NaCl, a peak containing a >99% pure fraction was obtained. Lanes: A, early fraction; B, later fraction of purified protein; MW, same molecular mass markers as in Fig. 1.

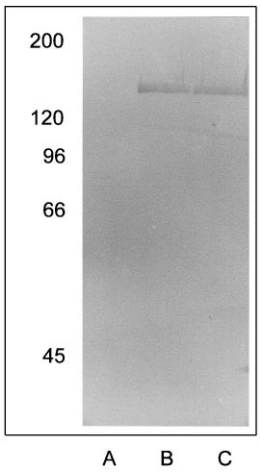

FIG. 3. Western blot of crude ECP from *S. epidermidis* M7 (lane A) and RP62A (lane B) and the purified 140-kDa protein (lane C). Proteins were blotted onto nitrocellulose membranes and probed with antiserum (1:50,000 dilution) raised in rabbits against the purified 140-kDa protein by using an alkaline phosphatase reaction. The values on the left are molecular masses in kilodaltons.

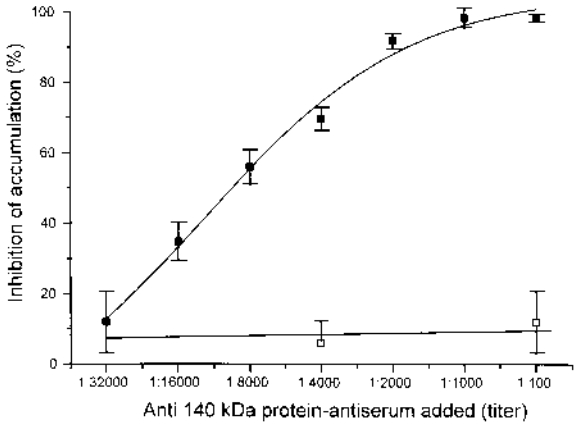

FIG. 5. Inhibition of accumulation of *S. epidermidis* RP62A on polystyrene. Microorganisms were suspended in CDM agar containing the indicated dilutions of antiserum raised against the purified 140-kDa protein and incubated for 2 h at 4°C and then for 18 h at 37°C. Inhibition of safranin-stainable biofilm formation was calculated as described in Materials and Methods. Symbols: ■, antiserum; □, preimmune serum. Shown are mean values ± standard deviations (triplicate determinations).

for the 140-kDa antigen did not form a biofilm (OD_{492}, 0.0 [0.0 to 0.85]), a difference found to be highly significant ($P < 0.001$; Mann-Whitney test) (Fig. 4). A similar relationship was found when accumulative growth was determined by using the glass tube test (6). Fifteen (46.8%) of 32 140-kDa antigen-positive isolates and only 3 (11.5%) of 26 140-kDa antigen-negative isolates formed a visible biofilm ($P = 0.05$; Fisher exact test).

Increasing concentrations of antiserum added to the medium containing RP62A inhibited biofilm formation in polystyrene microtiter plates and completely blocked accumulation with antiserum dilutions of ≤1:1,000 (Fig. 5). In wells containing liquid CDM, bacteria, and no antiserum, the OD_{492} was 1.84 ± 0.16 (mean ± standard deviation; $n = 3$), whereas the OD_{492} in the antibody-treated wells was 0.03 ± 0.02. Preimmune serum had no sizeable effect on accumulation (Fig. 5, open symbols). Similar results were obtained with the glass tube test. Whereas RP62A formed large amounts of biofilm (+++) both without serum and in the presence of preimmune serum (1:10 dilution), addition of antiserum at a concentration of 1:2,000 or higher completely inhibited visible biofilm formation on glass tubes. The specific antiserum inhibited accumulative growth of additional CoNS strains (Table 1). This effect of the antiserum was lost upon preadsorption of the antibody with 140-kDa antigen-positive strain Y14, whereas preadsorption of the antiserum with 140-kDa antigen-negative, biofilm-positive strain T5 did not change the effect of the antiserum on CoNS accumulation.

In addition to anti-140-kDa protein antibodies, inhibition of accumulation was tested by adding the purified 140-kDa antigen (up to 200 μg/ml) together with bacteria and CDM to the inoculum. No inhibition of biofilm formation was observed

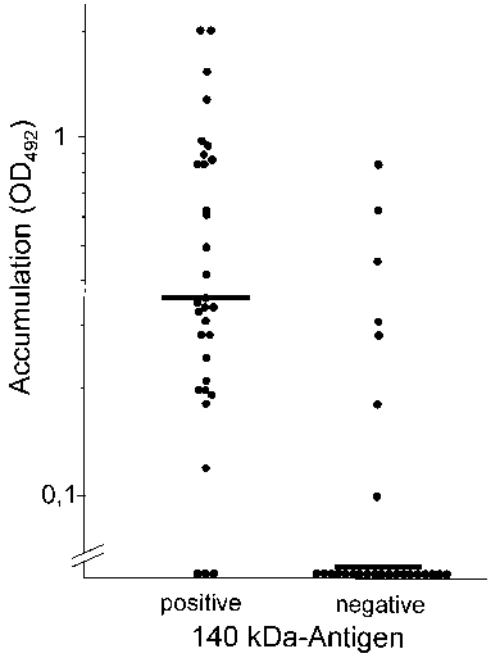

FIG. 4. Accumulation of 58 coagulase-negative clinical isolates on polystyrene. Presence or absence of the 140-kDa antigen was detected as described in the legend to Fig. 3, and the microtiter test (8), modified as described in Materials and Methods, was employed. The difference in OD_{492} between 140-kDa antigen-positive and -negative strains was found to be highly significant ($P < 0.001$; Mann-Whitney rank sum test).

TABLE 1. Effect of anti-140-kDa protein serum on biofilm formation of clinical CoNS strains[a]

Strain	OD_{492} (% inhibition)			
	Without antiserum	With antiserum preadsorbed with strain:		
		M7	T5	Y14
AB2	1.033	0.022 (98)	0.13 (95)	0.980 (5)
AB7	1.001	0.001 (99)	0.08 (98)	0.930 (7)
AD20	0.984	0.012 (99)	0.000 (100)	0.916 (7)
U3	0.263	0.002 (98)	0.000 (100)	0.225 (15)
T5	0.98	0.000 (100)	0.140 (85)	0.915 (7)

[a] 140-kDa protein-positive CoNS strains were inoculated and grown in polystyrene microtiter plates in the presence of the indicated preadsorbed antisera (dilution, 1:1,000) as described in Materials and Methods. After decanting, adherent growth was determined by measuring the OD_{492} of safranin-stained wells. M7, biofilm negative, 140-kDa antigen-negative mutant of RP62A; T5, biofilm-positive, 140-kDa antigen-negative strain; Y14, biofilm-negative, 140-kDa antigen-positive strain.

(data not shown). In contrast, accumulation was inhibitable if trypsin (100 μg/ml) or chymotrypsin (100 μg/ml) was added to the inoculum, as demonstrated by the tube test, while proteinase K had no effect (data not shown).

DISCUSSION

Accumulative growth on polymer surfaces resulting in biofilm formation is an important characteristic of *S. epidermidis*, contributing to pathogenicity by reducing the efficacy of host defenses (17, 27, 31, 49) and antimicrobial killing (15, 16, 47). In this report, biochemical and functional evidence for a role of a 140-kDa protein essential for accumulative growth of *S. epidermidis* RP62A is provided. (i) Accumulation of strain RP62A and of several clinical CoNS isolates is completely inhibited by antiserum directed against the purified 140-kDa ECP. The activity of the antiserum is lost upon preadsorption with a 140-kDa-positive CoNS strain. (ii) Biofilm formation is inhibited upon addition of proteases to the growth medium. (iii) The presence of the 140-kDa protein in crude extracellular material of strain RP62A can be demonstrated only by using sessile, not planctonic, growth conditions. (iv) The presence of 140-kDa proteins from different clinical and laboratory CoNS isolates and cross-reaction with anti-140-kDa antigen serum are significantly associated with accumulative growth; however, a few biofilm-positive but 140-kDa antigen-negative strains were also observed.

Several hypotheses may explain the failure of purified adhesion-associated protein (AAP) to inhibit accumulation. (i) AAP may serve as an anchor molecule on the staphylococcal surface binding to a complex polymeric substance such as polysaccharide intercellular adhesin (PIA) or polysaccharide/adhesin (PS/A) through multiple interactive sites on the carbohydrate, and it may thus be impossible to obtain sufficient concentrations of excess receptor for saturation of binding sites on the carbohydrate. (ii) The native form of the protein may be critical. (iii) The protein's effect may be indirect (e.g., as a signal transduction factor), requiring localization associated with intact membranes. The few 140-kDa antigen-negative, biofilm-producing strains observed in our study may possess similar proteins which do not share antigenicity with the 140-kDa protein, or they may provide other mechanisms to compensate for the apparent lack of the 140-kDa protein.

Several factors contributing to the adhesion of *S. epidermidis* to polymers have been identified. PS/A (53), which consists predominantly of galactose and glucosamine and has been characterized by G. Pier and coworkers in *S. epidermidis* RP62A, inhibits adhesion of RP62A to silastic and elicits adhesion-inhibiting antibodies. Transposon mutants deficient in PS/A did not adhere to silastic and also failed to accumulate (40). Subsequently, a role for PS/A as a CoNS virulence factor was demonstrated in various animal models of endovascular infections (29, 48, 50), and a correlation between clinical CoNS isolates expressing PS/A and invasive disease was demonstrated (41). In addition, two staphylococcal surface proteins of *S. epidermidis* 354 involved in adhesion have been described by Timmermann et al. (52) and Veenstra et al. (55). Antigenically related proteins are present on *S. epidermidis* RP62A, as demonstrated by using mAb36.4 (51). By using Tn*917* transposon insertion mutagenesis, Heilmann et al. generated *S. epidermidis* mutants that were deficient in primary adhesion and subsequent safranin-stainable biofilm formation on polystyrene but retained the ability to form a biofilm on glass; adhesion deficiency in these strains was associated with the loss of five cell surface proteins, and adhesion could be restored upon transformation with a gene conferring a 60-kDa protein (19). Thus, both carbohydrate and protein factors that influence adhesion have been identified.

In addition to adhesion, *S. epidermidis* must be able to accumulate on a surface when a biofilm is formed. Several previous attempts to elucidate the factors contributing to accumulation focused on analysis of the chemical nature of exopolymer substances derived from various *S. epidermidis* strains (2, 5, 33, 39, 45). However, in these studies complex carbohydrates were identified but their role in biofilm formation was not shown (30). Also, no differences in the chemical composition of slime material isolated from accumulation-positive or -negative strains have been demonstrated. Culture medium components have contaminated the exopolymers recovered from *S. epidermidis* (13, 25); thus, in later studies, care was taken to avoid contamination of the harvested material by growing *S. epidermidis* on dialysis membranes (45) laid over chemically defined medium (23, 24). With this approach, *S. epidermidis* exopolymers consisting mainly of teichoic acid (21) and various proteins were found, and their composition was also the same whether they came from biofilm-producing or non-biofilm-producing CoNS strains (25).

Further studies to define the factor(s) important for accumulation used an immunologic approach. Christensen et al. used a polyclonal antibody to detect a protein-carbohydrate mixture called slime-associated antigen (SAA) from strain RP62A. This was absent in two nonaccumulating spontaneous and acriflavin-induced variant strains (5) and in non-slime-producing clinical strains (3). Mack et al. used antiserum adsorption with nonaccumulating *S. epidermidis* strains (37) and have described a carbohydrate antigen important for accumulation and, by screening a transposon insertion library, have identified mutants specifically deficient in accumulation but unaffected in adhesion (36). This antigen has been analyzed as a β-1,6-linked glucosaminoglycan and has been termed PIA (35). The genetic basis for PIA production appears to be an *icaABC* gene cluster which has been recently identified by Heilmann et al. upon screening of an RP62A library expressed in *S. carnosus* for biofilm formation (20). *S. carnosus* carrying the plasmid pCN27 containing the *icaABC* gene cluster was found to be positive for PIA production and exhibits cell cluster formation. In addition to *S. carnosus*, *S. epidermidis* O-47-derived mutants with the ability to adhere but with a loss of accumulation ability (mut2 strains) (19) could be complemented with pCN27, resulting in restored PIA production and cell clustering (20). Recently, it has been suggested that SAA and PIA may be similar or identical in chemical composition (3).

Our 140-kDa antigen appears to be distinct from the aforementioned *S. epidermidis* factors involved in adhesion or accumulation. (i) Mutant M7 adheres equally well to polystyrene when compared to strain RP62A, as shown by CFU and ATP release measurements (46); thus, the expression of PS/A or SSP appears to be functionally unrelated to the 140-kDa protein. (ii) The 140-kDa protein is not expressed in biofilm-positive strain O-47 which contains five AAPs (including a 120-kDa protein) lost in mut1 strains. (iii) The biochemical characteristics of SAA as a complex antigen consisting mainly of glucose, amino sugars, and protein appear to be different from those of our (proteinaceous) antigen; however, the possibility that our 140-kDa protein is contained in SAA preparations cannot be excluded. (iv) The amounts of PIA produced by M7 and RP62A are identical (34), and carbohydrates are absent in our 140-kDa protein preparation. (v) The presence of the 140-kDa antigen can be demonstrated in neither *S. carnosus* nor *S. carnosus*(pCN27).

We conclude that the 140-kDa protein is a newly identified

accumulation-associated factor in *S. epidermidis* strains. While its exact biological function needs further elucidation, the protein appears to be unrelated to other *S. epidermidis* factors associated with biofilm formation, and therefore we propose to refer to it as AAP. Further molecular characterization of AAP is necessary to define its potential role in pathogenicity in vivo; however, the observations that this antigen is present on clinical isolates forming biofilms and that antibodies block accumulation may open exciting new avenues for immunotherapy in the prevention of *S. epidermidis* infections.

ACKNOWLEDGMENTS

This work was supported by a grant from the German Minister for Education and Research (grant 01 KI 9450) and a postdoctoral grant from the Alexander von Humboldt Foundation to M. Hussain.

We thank D. Mack for determination of PIA production and R. A. Proctor for critically reviewing the manuscript.

REFERENCES

1. **Ames, B.** 1966. Assay of inorganic phosphorus, total phosphorus and phosphates. Methods Enzymol. **8:**115–118.
2. **Arvanati, A., N. K. Karamanos, G. Dimitracopoulos, and E. D. Anastassiou.** 1994. Isolation and characterization of a novel 20 kDa sulfated polysaccharide from the extracellular slime layer of *Staphylococcus epidermidis*. Arch. Biochem. Biophys. **308:**432–438.
3. **Baldassari, L., G. Donelli, A. Gelosia, M. C. Voglino, A. W. Simpson, and G. D. Christensen.** 1996. Purification and characterization of the staphylococcal slime-associated antigen and its occurrence among *Staphylococcus epidermidis* clinical isolates. Infect. Immun. **64:**3410–3415.
4. **Christensen, G. D., L. Baldassari, and W. A. Simpson.** 1994. Colonization of medical devices by coagulase-negative staphylococci, p. 45–78. *In* A. L. Bisno and F. A. Waldvogel (ed.), Infections associated with indwelling medical devices. American Society for Microbiology, Washington, D.C.
5. **Christensen, G. D., L. P. Barker, T. P. Mawhinney, L. M. Baddour, and W. A. Simpson.** 1990. Identification of an antigenic marker of slime production for *Staphylococcus epidermidis*. Infect. Immun. **58:**2906–2911.
6. **Christensen, G. D., W. A. Simpson, A. L. Bisno, and E. H. Beachey.** 1982. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect. Immun. **37:**318–326.
7. **Christensen, G. D., W. A. Simpson, A. L. Bisno, and E. H. Beachey.** 1983. Experimental foreign-body infections in mice challenged with slime-producing *Staphylococcus epidermidis*. Infect. Immun. **40:**407–410.
8. **Christensen, G. D., W. A. Simpson, J. J. Younger, L. M. Baddour, F. F. Barrett, D. M. Melton, and E. H. Beachey.** 1985. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J. Clin. Microbiol. **22:**996–1006.
9. **Costerton, J. W., K.-J. Cheng, G. G. Geesey, T. I. Ladd, J. C. Nickel, M. Dasgupta, and T. J. Marrie.** 1987. Bacterial biofilms in nature and disease. Annu. Rev. Microbiol. **41:**435–464.
10. **Davenport, D. S., R. M. Masanari, M. A. Pfaller, M. J. Bale, S. A. Streed, and W. J. Hierholzer, Jr.** 1986. Usefulness of a test for slime production as a marker for clinically significant infections with coagulase-negative staphylococci. J. Infect. Dis. **153:**332–339.
11. **Deighton, M. A., and B. Balkau.** 1990. Adherence measured by microtiter assay as a virulence marker for *Staphylococcus epidermidis* infections. J. Clin. Microbiol. **28:**2442–2447.
12. **Diaz-Mitoma, F., G. K. M. Harding, D. J. Hoban, R. S. Roberts, and D. E. Low.** 1987. Clinical significance for slime production in ventriculoperitoneal shunt infections caused by coagulase-negative staphylococci. J. Infect. Dis. **156:**555–560.
13. **Drewry, D. T., L. Galbraith, B. J. Wilkinson, and S. G. Wilkinson.** 1990. Staphylococcal slime: a cautionary tale. J. Clin. Microbiol. **28:**1292–1296.
14. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rabers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28:**350–356.
15. **Evans, R. C., and C. J. Holmes.** 1987. Effect of vancomycin hydrochloride on *Staphylococcus epidermidis* biofilm associated with silicone elastomer. Antimicrob. Agents Chemother. **31:**889–894.
16. **Farber, B. F., M. H. Kaplan, and A. G. Clogston.** 1990. Staphylococcus epidermidis extracted slime inhibits the antimicrobial action of glycopeptide antibiotics. J. Infect. Dis. **161:**37–40.
17. **Gray, E. D., G. Peters, M. Verstegen, and W. E. Regelmann.** 1984. Effect of extracellular slime substance from *Staphylococcus epidermidis* on the human cellular immune response. Lancet **i:**365–367.
18. **Harlow, E., and D. Lane.** 1988. Antibodies. A laboratory manual, p. 53–138. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Heilmann, C., C. Gerke, F. Perdreau-Remington, and F. Götz.** 1996. Characterization of Tn*917* insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. Infect. Immun. **64:**277–282.
20. **Heilmann, C., O. Schweitzer, C. Gerke, N. Vanittanakom, D. Mack, and F. Götz.** 1996. Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. Mol. Microbiol. **20:**1083–1091.
21. **Hussain, M., C. Collins, J. G. M. Hastings, and P. J. White.** 1992. Radiochemical assay to measure the biofilm produced by coagulase-negative staphylococci on solid surfaces and its use to quantitate the effects of various antibacterial compounds on the formation of the biofilm. J. Med. Microbiol. **37:**62–69.
22. **Hussain, M., J. G. Hastings, and J. G. White.** 1992. Comparison of cell-wall teichoic acid with high-molecular-weight extracellular slime material from *Staphylococcus epidermidis*. J. Med. Microbiol. **37:**368–375.
23. **Hussain, M., J. G. M. Hastings, and P. J. White.** 1991. Isolation and composition of the extracellular slime made by coagulase-negative staphylococci in a chemically defined medium. J. Infect. Dis. **163:**534–541.
24. **Hussain, M., J. G. M. Hastings, and P. J. White.** 1991. A chemically defined medium for slime production by coagulase-negative staphylococci. J. Med. Microbiol. **34:**143–147.
25. **Hussain, M., M. H. Wilcox, and P. J. White.** 1993. The slime of coagulase-negative staphylococci: biochemistry and relation to adherence. FEMS Microbiol. Rev. **104:**191–208.
26. **Ishak, M. A., D. G. M. Gröschel, G. L. Mandell, and R. P. Wenzel.** 1985. Association of slime with pathogenicity of coagulase-negative staphylococci causing nosocomial septicemia. J. Clin. Microbiol. **22:**1025–1029.
27. **Johnson, G. M., P. E. Lee, W. E. Regelmann, E. D. Gray, G. Peters, and P. G. Quie.** 1986. Interference with granulocyte function by *Staphylococcus epidermidis* slime. Infect. Immun. **54:**13–20.
28. **Kloos, W. E., and T. E. Bannerman.** 1994. Update on clinical significance of coagulase-negative staphylococci. Clin. Microbiol. Rev. **7:**117–140.
29. **Kojima, Y., M. Tojo, D. A. Goldmann, T. D. Tosteson, and G. B. Pier.** 1990. Antibody to the capsular polysaccharide/adhesin protects rabbits against catheter-related bacteremia due to coagulase-negative staphylococci. J. Infect. Dis. **162:**435–441.
30. **Kotilainen, P., J. Maki, P. Oksman, M. K. Viljanen, J. Nikoskelainen, and P. Houvinen.** 1990. Immunochemical analysis of the extracellular slime of *Staphylococcus epidermidis*. Eur. J. Clin. Microbiol. Infect. Dis. **9:**262–270.
31. **Kristinsson, K. G., J. G. Hastings, and R. C. Spencer.** 1988. The role of extracellular slime in opsonophagocytosis of *Staphylococcus epidermidis*. J. Med. Microbiol. **27:**207–213.
32. **Levvy, G. A., and A. McAllan.** 1959. The N-acetylation and estimation of hexosamines. Biochem. J. **73:**127–132.
33. **Ludwicka, A., G. Ulenbruck, G. Peters, P. N. Seng, E. D. Gray, J. Jeljaszewicz, and G. Pulverer.** 1984. Investigation on extracellular slime substance produced by *Staphylococcus epidermidis*. Zentralbl. Bakteriol. Hyg. 1 Abt. Orig. A **258:**256–267.
34. **Mack, D.** 1995. Personal communication.
35. **Mack, D., W. Fischer, A. Krokotsch, K. Leopold, R. Hartmann, H. Egge, and R. Laufs.** 1996. The intercellular adhesin involved in biofilm accumulation of *Staphylococcus epidermidis* is a linear β-1,6-linked glucosaminoglycan: purification and structural analysis. J. Bacteriol. **178:**175–183.
36. **Mack, D., M. Nedelmann, A. Krokotsch, A. Schwarzkopf, J. Heesemann, and R. Laufs.** 1994. Characterization of transposon mutants of biofilm-producing *Staphylococcus epidermidis* impaired in the accumulative phase of biofilm production: genetic identification of a hexosamine-containing polysaccharide intercellular adhesin. Infect. Immun. **62:**3244–3253.
37. **Mack, D., N. Siemssen, and R. Laufs.** 1992. Parallel induction by glucose of adherence and a polysaccharide antigen specific for plastic-adherent *Staphylococcus epidermidis*. Infect. Immun. **60:**2048–2057.
38. **Marrie, T. J., and J. W. Costerton.** 1984. Scanning and transmission electron microscopy of in situ bacterial colonization of intravenous and intraarterial catheters. J. Clin. Microbiol. **19:**687–693.
39. **McDonald, H. C., and W. W. Karakawa.** 1970. Immunochemical analysis of uronic acid polymer of *Staphylococcus epidermidis* strain 53. J. Immunol. **105:**389–395.
40. **Muller, E., J. Hübner, N. Gutierrez, S. Takeda, D. A. Goldmann, and G. B. Pier.** 1993. Isolation and characterization of transposon mutants of *Staphylococcus epidermidis* deficient in capsular polysaccharide/adhesin and slime. Infect. Immun. **61:**551–558.
41. **Muller, E., S. Takeda, H. Shiro, D. Goldmann, and G. D. Pier.** 1993. Occurrence of capsular polysaccharide/adhesin among clinical isolates of coagulase-negative staphylococci. J. Infect. Dis. **168:**1211–1218.
42. **Patti, J. M., and M. Höök.** 1994. Microbial adhesins recognizing extracellular matrix macromolecules. Curr. Opin. Cell Biol. **6:**752–758.
43. **Peters, G., R. Locci, and G. Pulverer.** 1981. Microbial colonization of prosthetic devices. II. Scanning electron microscopy of naturally infected intravenous catheters. Zentralbl. Bakteriol. Hyg. 1 Abt. Orig. B **173:**293–299.
44. **Peters, G., R. Locci, and G. Pulverer.** 1982. Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. J. Infect. Dis. **146:**479–482.
45. **Peters, G., F. Schumacher-Perdreau, B. Jansen, M. Bey, and G. Pulverer.**

1987. Biology of *Staphylococcus epidermidis* extracellular slime, p. 15–32. *In* G. Pulverer, P. G. Quie, and G. Peters (ed.), Pathogenicity and clinical significance of coagulase-negative staphylococci. Gustav Fischer Verlag, Stuttgart, Germany.

46. **Schumacher-Perdreau, F., C. Heilmann, G. Peters, F. Götz, and G. Pulverer.** 1994. Comparative analysis of a biofilm forming *Staphylococcus epidermidis* strain and its adhesion-positive, accumulation-negative isogenic mutant. FEMS Microbiol. Lett. **117:**71–78.
47. **Sheth, N. K., T. R. Franson, and P. G. Sohnle.** 1985. Influence of bacterial adherence to intravascular catheters on in vitro antibiotic susceptibility. Lancet **ii:**1266–1268.
48. **Shiro, H., E. Muller, N. Gutierrez, S. Boisot, M. Grout, T. D. Tosteson, D. Goldmann, and G. B. Pier.** 1994. Transposon mutants of *Staphylococcus epidermidis* deficient in elaboration of capsular polysaccharide/adhesin and slime are avirulent in a rabbit model of endocarditis. J. Infect. Dis. **169:**1042–1049.
49. **Stout, R. D., Y. Li, A. R. Miller, and D. W. Lambe, Jr.** 1994. Staphylococcal glycocalyx activates macrophage prostaglandin E_2 and interleukin 1 production and modulates tumor necrosis factor alpha and nitric oxide production. Infect. Immun. **62:**4160–4166.
50. **Takeda, J., G. B. Pier, Y. Kojima, M. Tojo, E. Muller, T. D. Tosteson, and D. A. Goldman.** 1991. Protection against endocarditis due to *Staphylococcus epidermidis* by immunization with capsular polysaccharide/adhesin. Circulation **84:**2539–2546.
51. **Timmermann, C. P., J. M. Besnier, L. de Graaf, R. Torensma, A. J. Verkley, A. Fleer, and J. Verhoef.** 1991. Characterisation and functional aspects of monoclonal antibodies specific for surface proteins of coagulase-negative staphylococci. J. Med. Microbiol. **35:**65–71.
52. **Timmermann, C. P., A. Fleer, J. M. Besnier, L. de Graaf, F. Cremers, and J. Verhoef.** 1991. Characterization of a proteinaceous adhesion of *Staphylococcus epidermidis* which mediates attachment to polystyrene. Infect. Immun. **59:**4187–4192.
53. **Tojo, M., D. A. Yamashita, D. A. Goldman, and G. D. Pier.** 1988. Isolation and characterization of a capsular polysaccharide adhesin from *Staphylococcus epidermidis*. J. Infect. Dis. **157:**713–722.
54. **Van der Rijn, I., and R. E. Kessler.** 1985. Growth characteristics of group A streptococci in a new chemically defined medium. Infect. Immun. **27:**444–448.
55. **Veenstra, G. J. C., F. F. M. Cremers, H. Van Dijk, and A. Fleer.** 1996. Ultrastructural organization and regulation of a biomaterial adhesin of *Staphylococcus epidermidis*. J. Bacteriol. **178:**537–541.
56. **Younger, J. J., G. D. Christensen, D. L. Bartley, J. C. H. Simmons, and F. F. Barrett.** 1987. Coagulase-negative staphylococci isolated from cerebrospinal fluid shunts: importance of slime production, species identification, and shunt removal to clinical outcome. J. Infect. Dis. **156:**548–554.

Editor: V. A. Fischetti