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Parallel Induction by Glucose of Adherence and a Polysaccharide Antigen Specific for Plastic-Adherent *Staphylococcus epidermidis*: Evidence for Functional Relation to Intercellular Adhesion

DIETRICH MACK,* NICOLAUS SIEMSSEN, AND RAINER LAUFS

Institute for Medical Microbiology and Immunology, University Hospital Eppendorf, Martinistrasse 52, D-2000 Hamburg 20, Germany

Received 13 September 1991/Accepted 24 February 1992

The initial attachment and the accumulation of *Staphylococcus epidermidis* on polymer surfaces in multilayered cell clusters embedded in amorphous slime, which together lead to the plastic-adherent phenotype detected by the adherence assay used in this study, have been proposed to be major virulence factors of these bacteria. An antigen specific for plastic-adherent *S. epidermidis* strains was detected by an indirect immunofluorescence test using absorbed antiserum raised against the strongly plastic-adherent *S. epidermidis* 1457. A coagglutination assay was established, which allowed the quantitation of the antigen in bacterial extracts under different physiologic growth conditions. Expression of the antigen and of plastic adherence depended significantly on the presence of glucose in the growth medium. Parallel to increased plastic adherence, a 32- to 64-fold increase in the amount of the antigen was detected in bacterial extracts of cells grown in tryptone soya broth (TSB) compared with that in extracts of cells grown in TSB lacking glucose. A parallel time-dependent increase of plastic adherence and expression of the antigen was observed after stimulation by glucose of stationary-phase cultures of plastic-adherent *S. epidermidis* strains grown in TSB lacking glucose. The antigen consisted most probably of polysaccharide, because its immunologic reactivity was completely abolished by periodate oxidation but was resistant to protease digestion. A significant proportion of cells of plastic-adherent as compared with nonadherent *S. epidermidis* strains grown in TSB were located in large cell clusters exceeding 50 cells, which completely disintegrated after periodate oxidation of the cell preparations. Periodate oxidation of adherent bacterial films in situ led to release of the adherent cells from the plastic surface. These results strongly indicate a functional relation of the antigen to adherence of *S. epidermidis* to polymer surfaces, most probably by mediating intercellular adhesion of cells leading to accumulation in multilayered cell clusters.

An increasing incidence of nosocomial infections due to coagulase-negative staphylococci has been noted in recent years, especially in relation with prosthetic biomaterials like intravascular catheters, peritoneal dialysis catheters, cerebrospinal fluid shunts, prosthetic heart valves, and prosthetic joints (for a review, see reference 23).

In scanning electron microscopic studies of infected intravascular catheters, coagulase-negative staphylococci were demonstrated to adhere to catheter surfaces in large multilayered cell clusters embedded in an amorphous slime substance (4, 12, 18, 21). The competence of coagulase-negative staphylococci to attach to and their competence to accumulate on polymer surfaces while producing slime were therefore proposed to be major virulence factors of these bacteria.

Both attachment and accumulation on plastic surfaces together lead to a plastic-adherent phenotype as detected by the semiquantitative adherence assay (6) used in the present study.

The importance of plastic adherence for virulence was supported by several clinical studies, which revealed that coagulase-negative staphylococci which were plastic adherent as defined above were significantly more often related to significant infections than nonadherent strains (4, 8, 9, 11, 13, 26). Furthermore, several of these studies indicated that plastic adherence of coagulase-negative staphylococci correlated with a negative outcome of treatment using antibiotics alone without removal of the infected foreign body (8, 10, 26). However, it should be noted that significant clinical infections were also observed with coagulase-negative staphylococci which were not plastic adherent.

The study of the pathogenicity of coagulase-negative staphylococci, especially *Staphylococcus epidermidis*, has focused mainly on plastic adherence and slime production.

A polysaccharide antigen (PS/A) which is rich in galactose and which probably mediates the initial attachment of *S. epidermidis* strains to catheter surfaces was purified from *S. epidermidis* RP62A (25). PS/A was, however, also demonstrated with staphylococcal strains which were not plastic adherent (19) and therefore apparently lacked the competence to accumulate on the polymer surface in multiple layers. Therefore, additional factors differentiate *S. epidermidis* strains which are plastic adherent from those strains which are not.

Recently, an antigen (SAA) was described as an antigenic marker for plastic-adherent *S. epidermidis*; it gave single precipitin lines in double immunodiffusion of extracts of the strongly plastic-adherent *S. epidermidis* RP62A and anti-RP62A antiserum but was not detected in extracts of a nonadherent morphology variant of strain RP62A and an acriflavin-induced, nonadherent mutant of this strain (3). SAA was shown to be serologically distinct from PS/A (3).

In the present study we identified an antigen which is specific for plastic-adherent *S. epidermidis* and which is expressed in large amounts in parallel to the expression of a plastic-adherent phenotype under different growth condi-

* Corresponding author.

tions. We provide evidence that the expression of this antigen is functionally related to the intercellular adhesion of staphylococcal cells in large cell clusters. Therefore, this antigen could be of functional significance for the accumulation of *S. epidermidis* cells on polymer surfaces in multiple layers.

(Part of this work will appear in the doctoral thesis of N.S.)

MATERIALS AND METHODS

Bacteria. *S. epidermidis* 5179, 8400, 8669, 9142, 9225, 9896, 10160, 10248, 10333, 10730, and 10993 and *S. epidermidis* 1457 were isolated from blood cultures and an infected central venous catheter, respectively, from our clinical microbiology laboratory. Strains were identified as *S. epidermidis* by Gram stain, negative coagulase and positive catalase tests, and biotyping by APIStaph (API Systems, Montalieu-Vercieu, France). *S. epidermidis* RP62A (ATCC 35984) and RP12 (ATCC 35983) and *Staphylococcus hominis* RP14 (ATCC 35981) and SP2 (ATCC 35982) were obtained from G. Peters (University of Cologne, Cologne, Germany). *S. epidermidis* 8400, 9142, 10160, 10248, 10730, 10993, 1457, RP12, and RP62A were plastic adherent as determined in the adherence assay (see below) and, when grown in tryptone soya broth (TSB), displayed a positive coagglutination reaction with our absorbed antiserum. Strains 5179, 8669, 9225, 9896, SP2, and RP14 were nonadherent (optical density at 570 nm [OD_{570}] < 0.1) and did not coagglutinate with the reagent. *Staphylococcus aureus* Cowan I was obtained from J. Heesemann (Würzburg, Germany).

Growth of bacteria and preparation of extracts. Bacteria were inoculated into TSB lacking glucose prepared from tryptone (Oxoid, Basingstoke, England), neutralized soya peptone (Oxoid), NaCl, and dipotassium phosphate as indicated by the manufacturer and incubated with shaking at 37°C for 22 h. Two 9-cm-diameter plastic tissue culture dishes (Nunc, Roskilde, Denmark) were each inoculated with 100 μl of culture in 10 ml of TSB (Becton Dickinson, Cockeysville, Md.) or TSB lacking glucose and incubated at 37°C for 24 h except when stated otherwise. Bacterial cells were scraped from the surface with a disposable cell scraper (Costar, Cambridge, Mass.) and were collected by centrifugation. The medium was aspirated, and the cells were resuspended in 5 ml of phosphate-buffered saline. The cell-associated specific antigen was quantitatively extracted by sonicating twice for 30 s on ice by using a sonicator cell disruptor (Heatsystems-Ultrasonics Inc., Plainview, N.Y.). Aliquots were removed for the determination of cell density by measuring the OD_{578}s of appropriate dilutions. Bacterial cells were removed by centrifugation at 1,500 × *g* for 15 min. Extracts were clarified by centrifugation at 16,000 × *g* for 1 h at 4°C and stored at −20°C.

For the analysis of antigen induction by glucose and other carbohydrates, these were added to TSB lacking glucose at a final concentration of 0.25% (wt/vol) from filter-sterilized stock solutions.

Adherence measurement. A plastic-adherent phenotype of *S. epidermidis* isolates was determined with a semiquantitative adherence assay using 96-well tissue culture plates (Nunc), as described previously with a slight modification (6), to measure attachment and accumulation on the plastic surface. Overnight cultures of the strains in TSB lacking glucose were diluted 1:100 in TSB or TSB lacking glucose, and aliquots (200 μl) were inoculated in each well. After 20 to 24 h at 37°C the plates were washed four times with phosphate-buffered saline, the adherent cells were fixed with Bouin's fixative, and cells were stained with gentian violet. The OD_{570} of stained adherent bacterial films was quantitated with an automatic spectrophotometer (Behring, Marburg, Germany). Strains were tested in quadruplicate. Plastic-adherent strains were defined to have a mean OD_{570} greater than 0.1.

Preparation and absorption of antiserum. *S. epidermidis* 1457 was grown in TSB in plastic tissue culture dishes for 20 h at 37°C. The medium was aspirated, and the adherent bacteria were scraped off from the plastic surface into phosphate-buffered saline. Formaldehyde was added to a final concentration of 0.5% (vol/vol), and the suspension was gently shaken for 20 h. Cells were collected by centrifugation, suspended in phosphate-buffered saline at an OD_{578} of 1.0, and stored in small aliquots at −20°C.

After preimmune sera were obtained, rabbits were immunized three times a week by intravenous injection of 0.2 ml of the bacterial suspension for the first week, 0.4 ml for the second week, 0.8 ml for the third week, and 1.0 ml thereafter for 3 months. Rabbits were exsanguinated, and serum was stored at −20°C.

The antiserum was absorbed with formalin-fixed nonadherent *S. epidermidis* 5179 and 9896 grown in TSB (15) by using the equivalent of 50 ml of culture per ml of serum. Bacterial cells were sedimented twice at 16,000 × *g* in a minicentrifuge, and the absorbed antiserum was stored at −20°C.

As preliminary experiments revealed that the expression of antigens specific for plastic-adherent *S. epidermidis* was extremely glucose dependent, the antiserum was further absorbed with *S. epidermidis* 1457 grown in TSB lacking glucose prepared with tryptone (Difco, Detroit, Mich.) as described above. All experiments were performed with this antiserum absorbed with the two nonadherent *S. epidermidis* strains and *S. epidermidis* 1457 grown in TSB lacking glucose.

Coagglutination test for detection of specific antigen. A 10% (wt/vol) suspension of formalin-fixed and heat-treated *S. aureus* Cowan I in phosphate-buffered saline with 0.05% NaN_3 was prepared as described elsewhere (15). Absorbed antiserum (100 μl) was incubated with 1 ml of *S. aureus* suspension for 15 min at room temperature. Bacterial cells were sedimented and washed three times with phosphate-buffered saline containing 0.05% NaN_3, suspended in 10 ml of this buffer, and stored at 4°C.

For the coagglutination assay of antigen, aliquots (5 μl) of bacterial extracts were mixed with 25 μl of coagglutination reagent on microscope slides. Agglutination was evaluated after 3 min in bright light against a dark background. No coagglutination was observed when aliquots of uninoculated TSB or TSB lacking glucose were tested as controls.

Quantitation of antigen in bacterial extracts was performed by analysis of serial twofold dilutions of the extracts in phosphate-buffered saline. The antigen titers were defined as the highest dilutions displaying a positive coagglutination test.

Immunofluorescence microscopy. Bacteria were grown in TSB or TSB lacking glucose prepared with tryptone (Difco) for 22 h at 37°C with shaking. Bacterial suspensions were diluted in phosphate-buffered saline to an OD_{578} of 0.5, and aliquots (20 μl per field) were applied to immunofluorescence slides (Biomerieux, Marcy l'Etoile, France). Slides were dried, fixed with cold acetone, and stored at 4°C until use.

Aliquots (20 μl per field) of antiserum diluted 1:50 in phosphate-buffered saline were applied to slides. After 30

min at 37°C slides were washed three times with phosphate-buffered saline, fluorescein-conjugated anti-rabbit immunoglobulin G (Sigma, Munich, Germany) diluted in phosphate-buffered saline was applied, and slides were incubated for 30 min at 37°C. After washing, slides were mounted in 90% glycerol in phosphate-buffered saline, pH 8.6, containing 0.1% *p*-diphenyleneamine (14) and viewed with a Orthoplan fluorescence microscope (E. Leitz, Wetzlar, Germany).

Periodate oxidation and protease digestion of antigen. Aliquots (80 μl) of extracts of different *S. epidermidis* strains were supplemented with 20 μl of 0.2 M sodium *meta*-periodate or 20 μl of water and incubated at 4°C for 23 h. Ethylene glycol (6.6 μl) was added, and antigen titers were determined by coagglutination.

Antigen extracts of different *S. epidermidis* strains were incubated in a final volume of 100 μl containing 100 mM Tris-HCl, pH 8.0, and 1 mg of proteinase K or pronase (Serva, Heidelberg, Germany) per ml at 37°C for 23 h. Protease was omitted as a control. Finally, 10 μl of 100 mM phenylmethylsulfonyl fluoride was added, and antigen titers were determined by coagglutination.

RESULTS

Our strategy to identify antigens specific for plastic-adherent *S. epidermidis* strains was to absorb antiserum raised against the strongly plastic-adherent *S. epidermidis* 1457 grown in TSB in tissue culture dishes with nonadherent *S. epidermidis* strains and to analyze the resulting absorbed antisera in an indirect immunofluorescence test (IFT). As expected, the antiserum cross-reacted with all *S. epidermidis* strains tested, independent of a plastic-adherent phenotype (data not shown). After absorption of the antiserum with the nonadherent *S. epidermidis* 5179 and 9896 grown in TSB, a bright and characteristic staining pattern of plastic-adherent *S. epidermidis* strains grown in TSB was detected in IFT, whereas no immunofluorescence was observed with nonadherent *S. epidermidis* strains (Fig. 1A and C).

When *S. epidermidis* strains were grown in TSB lacking glucose, only very weak immunofluorescent staining of the cells was detected independent of a plastic-adherent phenotype; however, the typical bright staining observed with plastic-adherent *S. epidermidis* strains grown in TSB was not observed (Fig. 1B and D).

As the expression of the plastic-adherent phenotype of *S. epidermidis* in TSB depended heavily on the presence of glucose in the medium (4), the specificity of our absorbed antiserum for antigens related to the expression of the plastic-adherent phenotype of plastic-adherent *S. epidermidis* strains was further increased by absorption with the strongly plastic-adherent *S. epidermidis* 1457 grown in TSB lacking glucose.

When this threefold-absorbed antiserum was tested with plastic-adherent *S. epidermidis* strains grown in TSB, the typical, bright immunofluorescence pattern was observed as described above. The reactive material seemed to surround the cells, whereas the cells remained almost free of immunofluorescent staining (Fig. 1E and G). No reactivity in IFT was observed with nonadherent control strains (Fig. 1J and K). When plastic-adherent *S. epidermidis* strains were grown in TSB lacking glucose, no fluorescence of the staphylococcal cells was observed (Fig. 1F and H). No fluorescence of *S. epidermidis* strains used in this study was observed in an IFT using preimmune serum (data not shown). Therefore, the absorbed antiserum specifically reacted with a cell-associated antigen of plastic-adherent *S. epidermidis* strains.

Dependence of cell-associated antigen expression on glucose. The degree of adherence of plastic-adherent *S. epidermidis* strains grown in TSB lacking glucose supplemented with exogenously added glucose depended on the brand of the medium components. Higher OD_{570} values were reached in the adherence assay using TSB lacking glucose prepared with tryptone supplied from Oxoid than were reached with medium prepared with tryptone manufactured by Difco (data not shown). Therefore, all experiments described below using TSB lacking glucose were performed with medium prepared with tryptone from Oxoid. However, when plastic-adherent *S. epidermidis* strains grown in TSB lacking glucose (Oxoid) were examined by IFT, restriction of expression of the antigen was not as complete as shown above, with TSB lacking glucose prepared with tryptone obtained from Difco, but a few cells exhibited remaining fluorescent staining resembling the typical pattern seen in Fig. 1E and G (data not shown).

As the IFT yielded only qualitative data on the expression of the specific antigen on the cell surface during different conditions of growth, we established a coagglutination assay using *S. aureus* Cowan I and the absorbed antiserum. The reagent specifically coagglutinated with cell suspensions of the plastic-adherent *S. epidermidis* strains grown in TSB for 24 h, whereas no coagglutination was observed with the nonadherent *S. epidermidis* strains used in the present study and with uninoculated TSB. Coagglutination depended on specific antibody, because no coagglutination was observed with reagent prepared with preimmune serum.

Preliminary experiments revealed that the specific antigen could be extracted quantitatively from the cells by sonication (data not shown). Quantitation of the amount of extracted antigen was performed by coagglutination of serial dilutions of the extracts. The assay was reproducible using the same batch of reagent. A twofold decrease in coagglutination titers was observed when the antigen was extracted from only half the amount of cells (data not shown).

The adherence of *S. epidermidis* cells to tissue culture dishes was extremely stimulated when the cells were grown in TSB compared with that of cells grown in TSB lacking glucose (Fig. 2A).

A 32- to 64-fold increase in coagglutination titers was observed in extracts of nine plastic-adherent *S. epidermidis* strains grown in plastic tissue culture dishes in TSB compared with titers in extracts of cells grown in TSB lacking glucose (Fig. 2B). No expression of the antigen could be detected in extracts of three nonadherent control strains (Fig. 2B). The increase in the amount of antigen was not due merely to greater bacterial cell density, as amounts of cells grown in TSB exceeded those of cells grown in TSB lacking glucose at most threefold (data not shown).

Induction of cell-associated expression of antigen by different carbohydrates. We have analyzed the coordinate expression of the specific antigen and the induction of a plastic-adherent phenotype by carbohydrates other than glucose with four strongly plastic-adherent *S. epidermidis* strains, 1457, 9142, RP62A, and 8400 (Fig. 3).

Plastic adherence was stimulated by D-glucose, fructose, D-galactose, saccharose, lactose, and maltose, whereas cellobiose, D-xylose, sorbitol, and α-arabinose did not lead to increased adherence (Fig. 3). Interestingly, plastic adherence of *S. epidermidis* 1457 was not stimulated by galactose and lactose (Fig. 3). This strain did not produce acid from lactose. As has been noted previously, lower degrees of

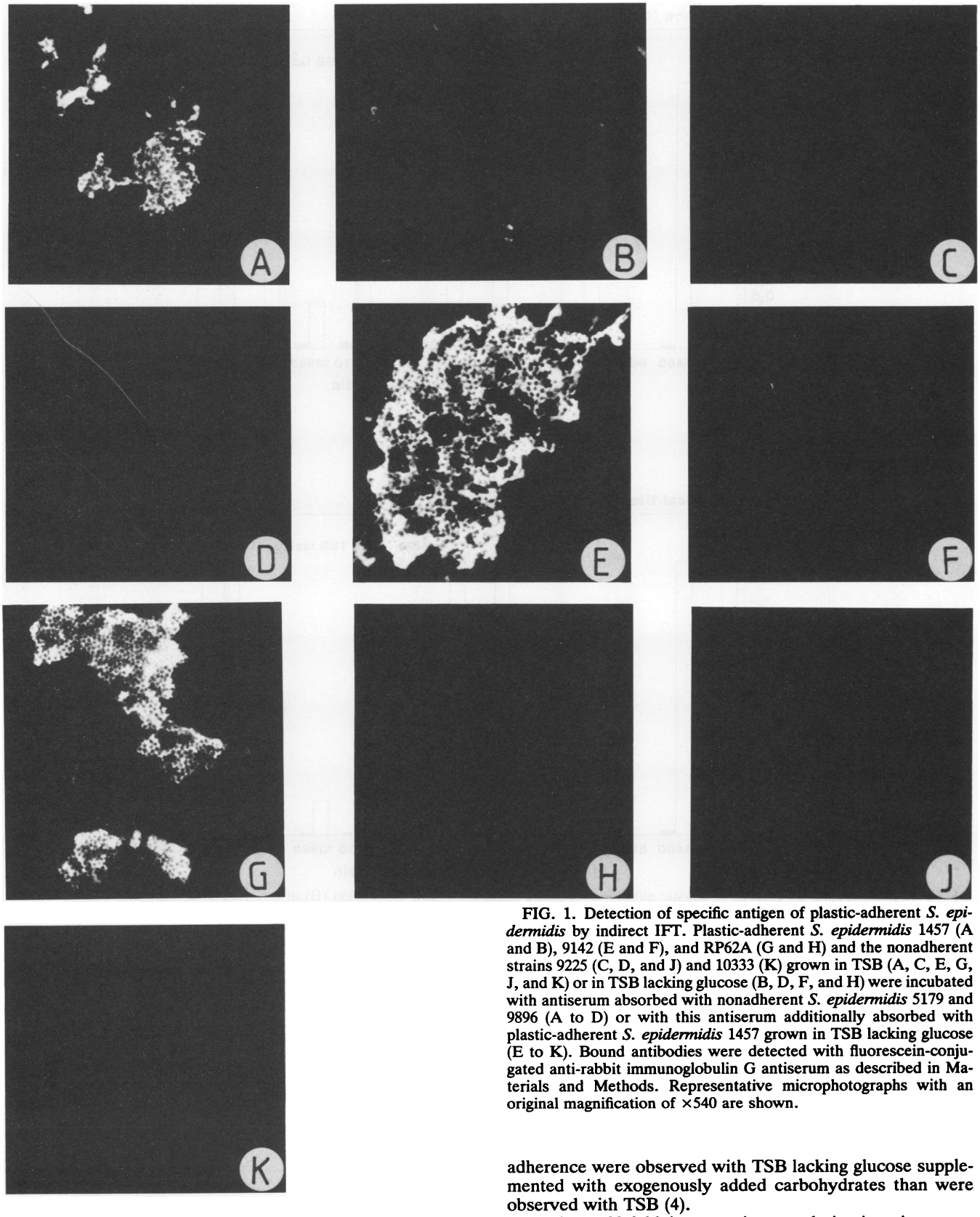

FIG. 1. Detection of specific antigen of plastic-adherent *S. epidermidis* by indirect IFT. Plastic-adherent *S. epidermidis* 1457 (A and B), 9142 (E and F), and RP62A (G and H) and the nonadherent strains 9225 (C, D, and J) and 10333 (K) grown in TSB (A, C, E, G, J, and K) or in TSB lacking glucose (B, D, F, and H) were incubated with antiserum absorbed with nonadherent *S. epidermidis* 5179 and 9896 (A to D) or with this antiserum additionally absorbed with plastic-adherent *S. epidermidis* 1457 grown in TSB lacking glucose (E to K). Bound antibodies were detected with fluorescein-conjugated anti-rabbit immunoglobulin G antiserum as described in Materials and Methods. Representative microphotographs with an original magnification of ×540 are shown.

adherence were observed with TSB lacking glucose supplemented with exogenously added carbohydrates than were observed with TSB (4).

An 8- to 32-fold increase in coagglutination titers was observed in bacterial extracts with carbohydrates which stimulated plastic adherence compared with titers in extracts

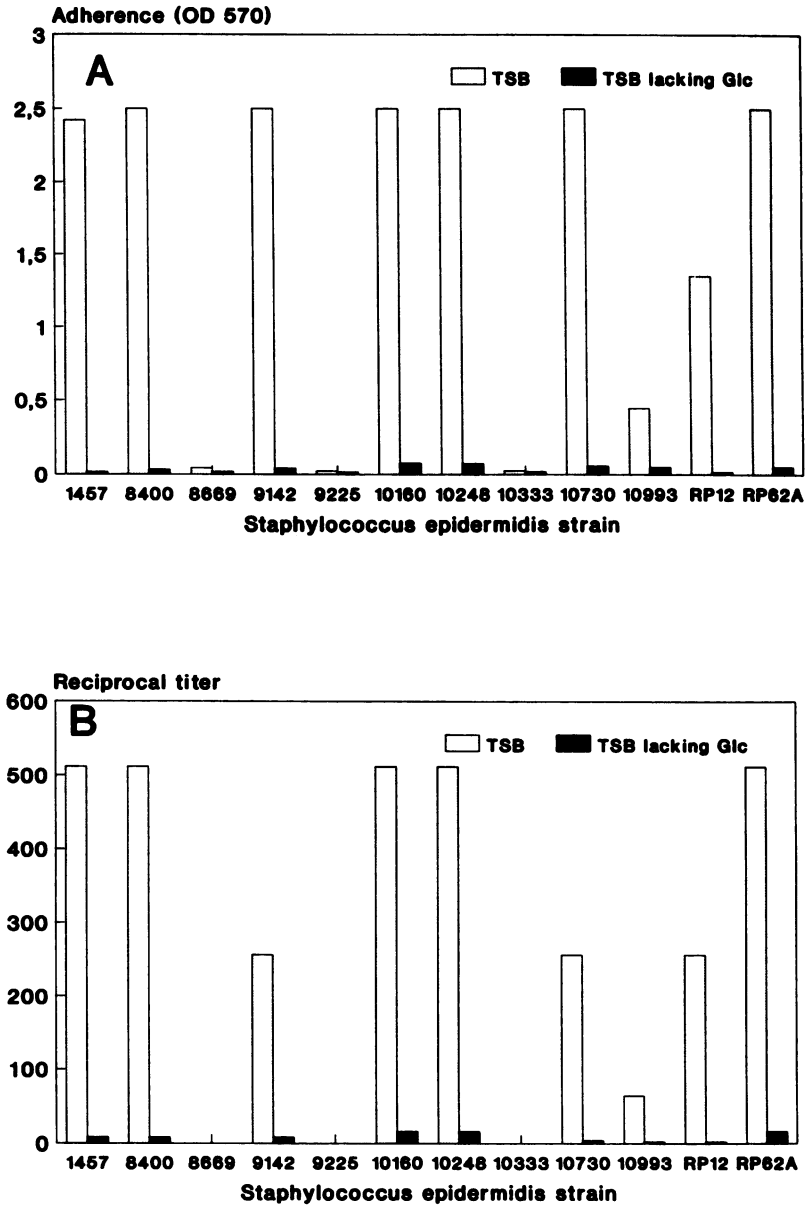

FIG. 2. Dependence on glucose of plastic adherence (A) and specific antigen expression (B) of different *S. epidermidis* strains. Plastic adherence of and the amount of antigen extracted from plastic-adherent *S. epidermidis* 1457, 8400, 9142, 10160, 10248, 10730, RP12, and RP62A and nonadherent strains 8669, 9225, and 10333 grown in TSB or in TSB lacking glucose in plastic tissue culture dishes were determined as described in Materials and Methods.

of the controls lacking any added carbohydrate (Fig. 3). Strain 1457 did not produce increased amounts of antigen when stimulated with galactose or lactose (Fig. 3). The increase in the amount of antigen was not due merely to greater bacterial cell density, as amounts of cells grown in carbohydrate-supplemented TSB exceeded those of cells grown in TSB lacking glucose only twofold.

Time dependence of induction of specific antigen and plastic adherence by glucose. To further investigate the linkage between expression of the cell-associated antigen and a plastic-adherent phenotype, the time dependence of expression of these two characteristics was analyzed after stimulation of stationary-phase cultures by addition of glucose to *S. epidermidis* 1457, 9142, RP62A, and 8400 grown in tissue culture dishes for 15 h in TSB lacking glucose.

Only a twofold increase in the amount of cells as determined by measuring the OD_{578} was observed when glucose was added 12 h before cell harvest compared with the amount of the nonstimulated control (data not shown).

The kinetic increase of plastic adherence was measured by inducing *S. epidermidis* cells grown in 96-well tissue culture trays for 15 h in TSB lacking glucose and adding glucose to 0.25% (final concentration) at different times before cell harvest. Adherence of cells as measured as stained bacterial films on the bottom of the tissue culture trays increased steadily with increasing time of stimulation with glucose. At

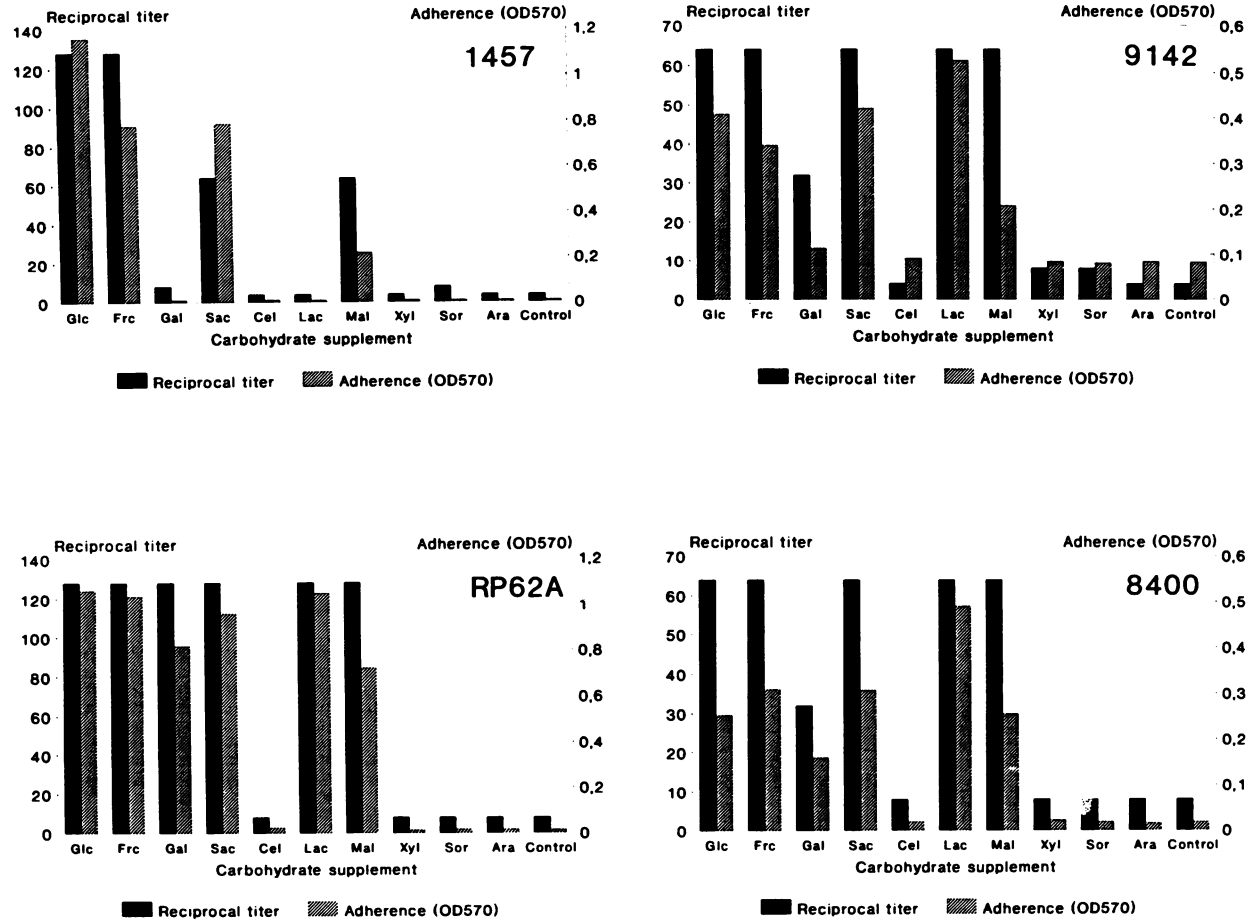

FIG. 3. Plastic adherence and antigen expression of plastic-adherent *S. epidermidis* 1457, 9142, RP62A, and 8400 grown in TSB lacking glucose supplemented with glucose (Glc), fructose (Frc), D-galactose (Gal), saccharose (Sac), cellobiose (Cel), lactose (Lac), maltose (Mal), xylose (Xyl), sorbitol (Sor), α-arabinose (Ara), or no added carbohydrate (Control). Plastic adherence and the amount of antigen in bacterial extracts of cells grown in single 9-cm-diameter plastic tissue culture dishes were determined as described in Materials and Methods.

8 to 10 h OD_{570} values comparable with those of cells grown in TSB supplemented with glucose for the entire growth period were reached (Fig. 4A).

The amount of antigen determined by coagglutination of cell extracts significantly increased after 4 h of stimulation with glucose, reaching maximal titers after 8 to 10 h of stimulation (Fig. 4B). At 12 h antigen titers had increased 8- to 32-fold compared with those of the unstimulated control and were comparable with titers of cells stimulated with glucose for the entire incubation time (Fig. 4B).

Periodate oxidation and protease digestion of antigen-containing extracts. To elucidate the nature of the antigen detected by our absorbed antiserum, extracts of plastic-adherent *S. epidermidis* 1457, 8400, 9142, 10160, 10248, RP62A, 10730, 10993, and RP12 were oxidized with sodium *meta*-periodate, which specifically destroys sugars containing unsubstituted dihydroxyl groups, or were incubated with proteinase K and pronase. The bacterial extracts had reciprocal antigen titers of 2,048 (strains 1457, 8400, 9142, 10160, and 10248), 4,096 (strain RP62A), 1,024 (strain 10730), 512 (strain 10993), and 256 (strain RP12). Antigen titers remained unchanged after incubation with the proteases for 23 h at 37°C, whereas no detectable coagglutinating activity was detected after periodate oxidation.

Release of adherent bacterial films by periodate oxidation. As the immunologic reactivity of the antigen of plastic-adherent *S. epidermidis* strains specifically detected by our absorbed antiserum was completely abolished by periodate oxidation, we investigated whether periodate oxidation would also have a functional effect on adherence. To this end the culture medium of adherent bacterial films of four strongly plastic-adherent *S. epidermidis* strains, 1457, 9142, RP62A, and 8400, grown in 96-well tissue culture trays in TSB for 22 h was aspirated, and the adherent cells were treated with 40 mM sodium *meta*-periodate, 40 mM sodium iodate, 40 mM KCl, 40 mM EDTA (pH 8.0), and 40 mM sodium borate (pH 8.0) for 23 h at 4°C. The adherent bacterial films were stained and quantitated as described in Materials and Methods. Oxidation of bacterial films with sodium *meta*-periodate led to almost complete release of the adherent bacterial films from the plastic surface (about 2% OD_{570} of the control incubated with phosphate-buffered saline), whereas the other agents had no significant effect on the release of adherent bacterial cells.

Similarly, the effect of protease digestion on the release of adherent bacterial films of the four *S. epidermidis* strains was studied with proteinase K, pronase, and trypsin. After 22 h of growth in TSB in 96-well tissue culture trays, the medium was aspirated. Aliquots (100 μl) of 100 mM Tris-HCl, pH 8.0, containing 10 mg of the respective proteases

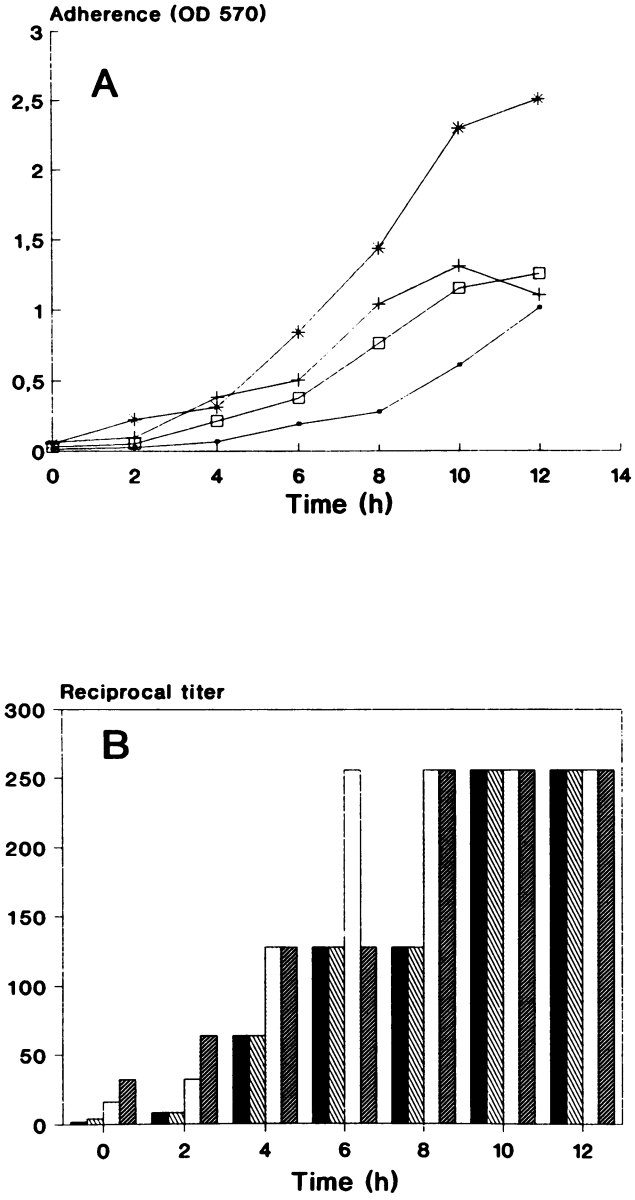

FIG. 4. Kinetic analysis of plastic adherence and antigen expression after induction with glucose. Plastic-adherent *S. epidermidis* 1457 (• and ■), 9142 (+ and ▨), RP62A (* and □), and 8400 (□ and ▨) grown in 96-well tissue culture plates for 15 h in TSB lacking glucose were induced by addition of glucose 12, 10, 8, 6, 4, and 2 h before termination of the experiment. (A) Plastic adherence was determined as described in Materials and Methods. (B) Extracts were prepared from the four *S. epidermidis* strains grown as described above, and amounts of antigen expressed were determined by coagglutination as described in Materials and Methods.

per ml were applied per well. The cells were incubated at 37°C for an additional 5 h, and the adherent bacterial films were quantitated as described in Materials and Methods. No significant release of adherent cells was observed with any of the three proteases (data not shown).

Cell cluster formation of plastic-adherent *S. epidermidis* strains. When the plastic-adherent *S. epidermidis* strains used in the present study were grown on plastic tissue culture dishes in TSB and were then scraped from the surface and suspended in phosphate-buffered saline, the cell suspensions were very sticky, as was previously noted (6). When appropriate dilutions of these suspensions were applied to microscope slides, Gram-stained preparations showed a significant proportion of the bacterial cells to be located in large cell clusters containing more than 50 cells (Fig. 5A). No such clusters were observed with the nonadherent, antigen-negative strains investigated (Fig. 5C).

When the cells were oxidized by addition of 40 mM sodium *meta*-periodate (final concentration) for 23 h at 4°C, the clusters observed with the plastic-adherent strains disappeared and the morphologies of the Gram-stained preparations were indistinguishable from those of nonadherent *S. epidermidis* strains (Fig. 5B and D). In parallel, the positive coagglutination reaction of the suspensions of plastic-adherent *S. epidermidis* strains became negative after periodate oxidation (data not shown). In addition, the bright fluorescent staining of the specific antigen of the plastic-adherent cells was no longer detectable after periodate oxidation (Fig. 5E and F).

DISCUSSION

Electron microscopic studies of infected intravascular catheters revealed that staphylococcal cells adhered to the catheter surfaces in large, multilayered cell clusters, which were cemented together by an amorphous material which was called slime (4, 12, 18, 21). When adherence of coagulase-negative staphylococci to catheters was studied in vitro, rapid attachment of staphylococcal cells to the polymer surface was followed by multiplication of bacterial cells and elaboration of amorphous slime material, engulfing the adherent cells in several layers, similar to what was seen in in vivo-infected catheters (22). Therefore, the competence to attach to polymer surfaces and competence to colonize polymer surfaces while producing slime were proposed to be major virulence factors of coagulase-negative staphylococci.

In the present study we identified an antigen specific for plastic-adherent *S. epidermidis* strains, i.e., strains competent to attach to and to accumulate on a plastic surface, in an indirect IFT, whereas several nonadherent *S. epidermidis* strains were negative for expression of this antigen. We developed a coagglutination test specific for the antigen, allowing the quantitation of expression of bacterial cell-associated antigen in bacterial extracts in a variety of physiologic growth conditions. The positive coagglutination reaction of cell suspensions and of extracts of plastic-adherent *S. epidermidis* strains depended on specific antibody and was not due to a possible intercellular adhesive property mediated by the specific antigen, because no coagglutination was observed with reagent prepared with preimmune serum.

The immunologic reactivity of the antigen in extracts of several strains of plastic-adherent *S. epidermidis* was unimpaired by digestion with proteinase K and pronase, whereas the reactivity was completely abolished by periodate oxidation (see above). As the antigen is retained by ultrafiltration membranes with a cutoff of 30 kDa (data not shown), these results suggest that the antigen is most probably composed of polysaccharide.

It has been previously shown that expression of a plastic-adherent phenotype of *S. epidermidis* strains depended extremely on the presence of glucose in the growth medium and that cells grown in TSB lacking glucose were only weakly adherent or nonadherent (4). Several carbohydrates, like D-fructose, D-galactose, saccharose, lactose, and maltose, could substitute for glucose in the enhancement of

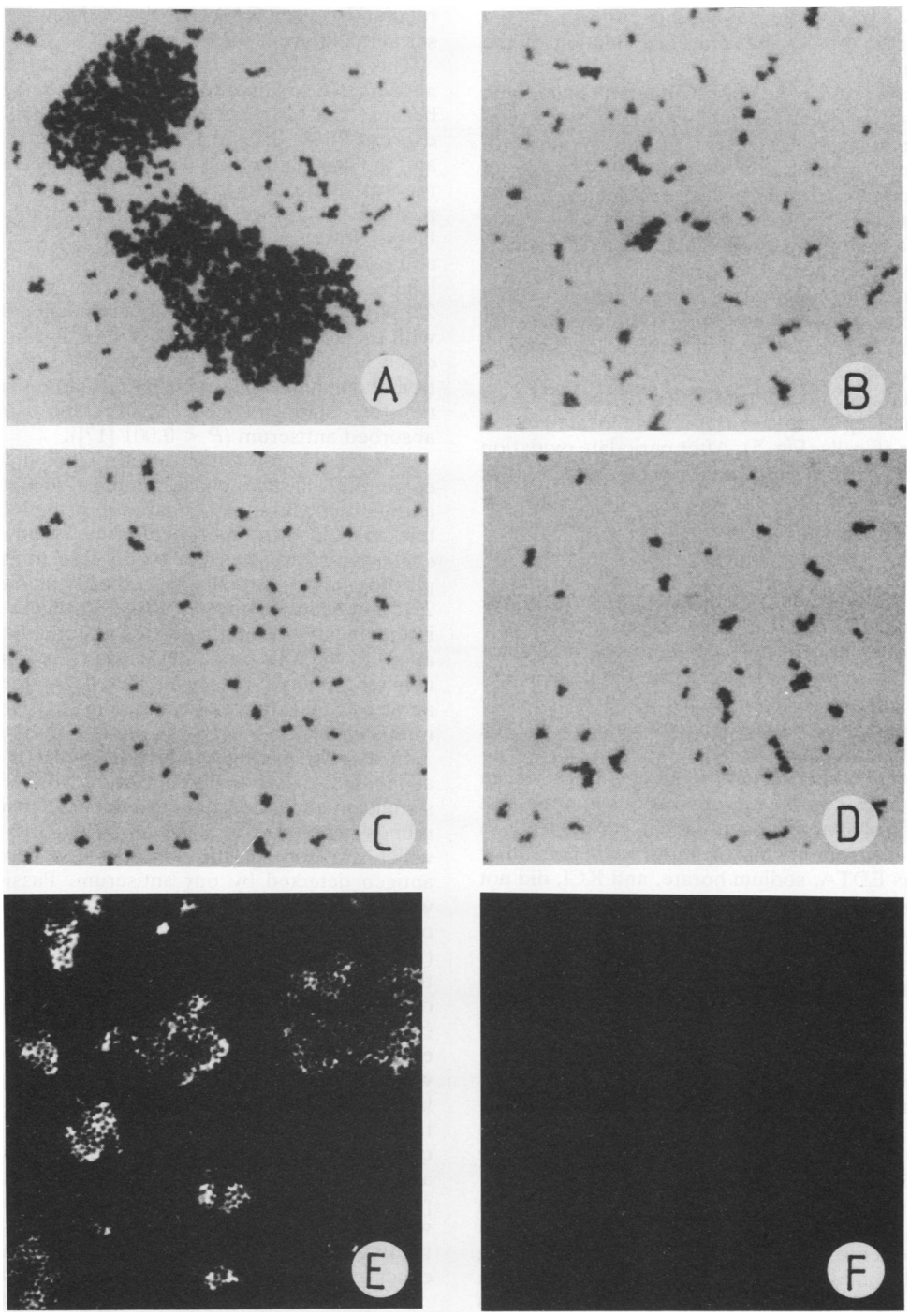

FIG. 5. Relation of cell clusters of plastic-adherent *S. epidermidis* strains to antigen expression. Plastic-adherent *S. epidermidis* 9142 (A, B, E, and F) and the nonadherent strain 9225 (C and D) were grown on plastic tissue culture dishes in TSB. Cells were scraped from the surface into phosphate-buffered saline and were oxidized with sodium *meta*-periodate for 23 h at 4°C (B, D, and F) or served as controls (A, C, and E). Shown are representative photomicrographs of Gram-stained preparations (A to D) and of an IFT after incubation with the threefold-absorbed antiserum (E and F) as described in Materials and Methods.

plastic adherence. However, other carbohydrates, like cellobiose, D-xylose, and α-arabinose, could not stimulate plastic adherence (4). In confirmation of these results, we show here in parallel with increased adherence an 8- to 64-fold increase in the amount of cell-associated antigen with different *S. epidermidis* strains grown in TSB and in TSB supplemented with glucose and the other carbohydrates stimulating adherence when compared with antigen with cells grown in TSB lacking glucose (Fig. 2 and 3). Increased amounts of cell-associated antigen were not detected with carbohydrates which did not lead to increased adherence of *S. epidermidis* cells. These experiments revealed a close

linkage of expression of large amounts of antigen on the bacterial surface and attachment and accumulation of the cells on plastic surfaces.

The parallel expression of a plastic-adherent phenotype and the specific antigen was further highlighted by a concomitant increase of adherence and of the amounts of cell-associated antigen after stimulation with glucose of stationary-phase cultures of four plastic-adherent *S. epidermidis* strains grown in TSB lacking glucose (Fig. 4). These results indicated a quantitative association between adherence and amounts of antigen.

These results are highly suggestive for the hypothesis that the antigen detected by our antiserum is functionally involved in attachment and accumulation of *S. epidermidis* cells on plastic surfaces.

We observed that a significant proportion of bacterial cells of plastic-adherent *S. epidermidis* strains were located in cell clusters exceeding 50 cells (Fig. 5). After periodate oxidation of such cell preparations, which destroyed the immunologic reactivity of the antigen, cell clusters were no longer detected and the morphology of these cell preparations resembled that of nonadherent control cells (Fig. 5). This result strongly indicates that the antigen may function as an adhesin between staphylococcal cells, leading to cell clustering and multilayered colonization of polymer surfaces.

Similarly, clustering of cells has been detected in *Streptococcus mutans* strains, which aggregated in the presence of sucrose by producing a polymeric glucan which specifically linked the cells together by interaction with a glucan-binding protein on the cell surface (7, 20, 24).

The function of the periodate-sensitive antigen as an intercellular adhesin is consistent with our observation that periodate oxidation of adherent bacterial films in situ leads to release of these cells from the plastic surface whereas other chemicals, such as EDTA, sodium borate, and KCl, did not lead to a significant release of adherent cells. No accessible proteins are apparently involved in stabilization of adherent bacterial films, because protease digestion did not result in a significant release of adherent bacteria from the plastic surface.

As the antigen reactive with our antiserum has not yet been purified, it cannot be completely excluded that the antiserum reacts with more than a single antigen. However, this seems quite unlikely, because the additional antigen had to be specific for plastic-adherent *S. epidermidis* and had to be coordinately regulated under the different physiologic growth conditions which were investigated.

An antigen (SAA) which gave single precipitin lines in double immunodiffusion of lysozyme-lysostaphin extracts of three plastic-adherent *S. epidermidis* strains, including RP62A and RP12, and anti-RP62A antiserum has been described previously but was not detected in similar extracts of the nonadherent *S. epidermidis* strain KH11 and two nonadherent *S. hominis* strains (3). The assumption that SAA is specifically related to plastic adherence of *S. epidermidis* strains is based mainly on the observation that SAA was also not detected in extracts of a nonadherent morphology variant of strain RP62A and an acriflavin-induced, nonadherent mutant of this strain (3). However, pleiotropic variations have been reported for morphology variants of RP62A with an altered adherence phenotype (1). Therefore, additional experimental evidence with additional *S. epidermidis* strains is needed to evaluate the relationship of SAA to plastic adherence. As the antigen detected by our antiserum has not yet been purified, the elucidation of a possible relation between SAA and the antigen defined by our antiserum must await further studies.

The previously described polysaccharide adhesin (PS/A) appears to be distinct from the antigen detected in this study, because the reference strain RP14, which was reported to express PS/A, did not react with our antiserum in the IFT and the coagglutination test. This result is supported by the report that most clinical isolates of *S. epidermidis* were PS/A positive, i.e., were able to rapidly attach to catheter surfaces. However, no association of PS/A expression with the ability to accumulate in multiple layers on polymer surfaces leading to a plastic-adherent phenotype was observed (19, 25). In contrast, a plastic-adherent phenotype as detected with the adherence assay described in Materials and Methods was significantly associated with a positive coagglutination reaction of cell suspensions of more than 200 coagulase-negative staphylococcal isolates by using our specific, absorbed antiserum ($P < 0.001$ [17]).

Apparently, the colonization of polymer surfaces by *S. epidermidis* has two phases: initial attachment to the surface and multiplication and generation of multilayered cell clusters embedded in amorphous slime. Many, if not most, *S. epidermidis* strains seem to be able to directly attach to plastic surfaces, probably specifically mediated by PS/A (19, 25). The data presented above strongly suggest that the intercellular adhesion, which is a prerequisite for the generation of multilayered cell clusters leading to a plastic-adherent phenotype, is mediated by the polysaccharide antigen specific for plastic-adherent *S. epidermidis* described in this study.

In a rabbit catheter infection model it was shown that active and passive immunization with PS/A leads to a significant reduction of the number of bacteremic days in rabbits infected with *S. epidermidis* RP12 and RP62A (16), which are both plastic adherent and express the specific antigen detected by our antiserum. Passive immunization with antiserum raised against PS/A inhibited the colonization of a second catheter inserted into the opposite jugular vein (16). Therefore, interference with the initial attachment to the polymer surface of *S. epidermidis* positive for expression of PS/A favorably influenced the course of experimental catheter infections in rabbits. A similar effect would be expected with *S. epidermidis* strains positive for PS/A, which although competent for attachment cannot accumulate on the polymer surface in multiple layers and are therefore not plastic adherent. Experimental evidence from catheter infection models in mice indicated that plastic-adherent coagulase-negative staphylococci had a greater pathogenic potential than nonadherent strains (2, 5). This was also suggested by several epidemiologic studies investigating the pathogenicity of coagulase-negative staphylococci in significant infections (4, 8, 9, 11, 13, 26).

Further studies will be needed to elucidate the individual and synergistic contributions of the antigen described in the present study and of PS/A to the pathogenicity of *S. epidermidis* in foreign body infections.

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