CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Nov. 1997, p. 705–710 Vol. 4, No. 6
1071-412X/97/$04.00+0

Phagocytosis and Oxidative-Burst Response of Planktonic *Staphylococcus epidermidis* RP62A and Its Non-Slime-Producing Variant in Human Neutrophils

MICHAEL HEINZELMANN, DANIEL O. HERZIG, BRIAN SWAIN, MARK A. MERCER-JONES, THOMAS M. BERGAMINI, AND HIRAM C. POLK, JR.*

Department of Surgery, Price Institute of Surgical Research, University of Louisville School of Medicine, Louisville, Kentucky 40292

Received 24 March 1997/Returned for modification 4 June 1997/Accepted 21 August 1997

The ability of bacterial organisms to produce an extracellular polysaccharide matrix known as slime has been associated with increased virulence and delayed infections in various prosthetic implants. Within a biofilm, this slime may protect the embedded bacteria from host defense mechanisms, especially phagocytosis by polymorphonuclear leukocytes. To determine whether planktonic *Staphylococcus epidermidis* is protected in a similar way, a novel flow cytometric assay was performed, measuring ingestion and adherence during phagocytosis and the production of superoxide during oxidative burst. Hydrophobicity was determined by hydrophobic interaction chromatography. Slime-producing *S. epidermidis* RP62A and its phenotypic variant, non-slime-producing RP62A-NA, were compared. The results showed increased phagocytosis of RP62A at 2, 5, 10, and 30 min; increased adherence of RP62A at 30 s and 30 min; and increased superoxide production of RP62A after 2 min. Decreased hydrophobicity of RP62A over RP62A-NA was correlated with a hydrophilic slime coat. The data argue that the host aggressively combats slime-producing *S. epidermidis*. This biological phenomenon is potentially important during bacteremia to prevent further adhesion, accumulation, and the genesis of a bacterial biofilm on implants or tissue surfaces.

The ability of bacterial organisms to produce an extracellular polysaccharide matrix known as slime has been associated with increased virulence and delayed infections in various prosthetic implants (8, 19, 21). The formation of (slime-containing) biofilms on surfaces can be considered a universal bacterial strategy for survival and for optimum positioning with regard to available nutrients. There is ample evidence that the presence of a biofilm may protect the embedded bacteria against host defense mechanisms, especially phagocytosis by polymorphonuclear leukocytes (PMNs) and antibiotic killing (8, 10, 33).

The clinical importance and pathogenic role of coagulase-negative staphylococci were first recognized in the early 1960s in infections of cerebrospinal fluid shunts (3, 26). Since then, biomaterial-centered infections with *Staphylococcus epidermidis* have emerged as a serious problem associated with the increased use of various biomaterial implants (1, 8, 31). *S. epidermidis* infections begin with rapid primary adherence of bacteria to exposed surfaces, followed by a longer-lasting phase of surface accumulation, with production of copious amounts of extracellular slime (21).

Phagocytosis of bacteria and the subsequent production of reactive oxygen intermediates (ROI) during oxidative burst represent an essential element of the host defense system in fighting microbial invasion. PMNs are considered to be professional phagocytes that function in concert with humoral components such as complement or immunoglobulins. Opsonization of bacteria by those components facilitates phagocytosis via complement and/or immunoglobulin Fc receptors (35). Phagocytosis is a complex process that can be divided into at least two major steps, adherence and internalization, with subsequent bacterial killing.

Many studies of slime-producing bacteria have used models with bacteria or bacterial biofilms adherent to a surface (7, 12, 20) or models in which extracellular slime preparations were added to non-slime-producing bacteria (10, 12, 23). Some studies have analyzed the difference between planktonic (i.e., suspended) and adherent bacteria. Riber et al. (22) showed that planktonic *S. epidermidis* induced one to three times more superoxide anion production than did adherent bacteria. Vergères and Blaser (33) demonstrated that antibiotic killing of adherent bacteria determined after 6 h was significantly reduced compared to that of planktonic bacteria. Kristinsson et al. (14) examined 43 isolates (slime positive and slime negative) of planktonic *S. epidermidis* for opsonophagocytosis. Their results indicate that slime did not change the requirement for opsonization or the efficiency of opsonization.

The goal of this study was to investigate the differences in phagocytosis and ROI production between *S. epidermidis* RP62A and its phenotypic non-slime-producing variant, RP62A-NA, in suspension. We used isolated human PMNs and a novel flow cytometric method to address this question. We show herein that the planktonic slime-producing variant of *S. epidermidis* demonstrates increased phagocytosis, adherence, and ROI production by human PMNs compared with its non-slime-producing phenotypic variant, RP62A-NA.

MATERIALS AND METHODS

Bacteria. *S. epidermidis* RP62A (ATCC 35984; American Type Culture Collection, Rockville, Md.) is a consistent slime producer when grown in tryptic soy broth (TSB). The phenotypic variant RP62A-NA does not produce slime when grown in TSB (6). Both bacteria were a kind gift from G. D. Christensen. *S. epidermidis* was grown overnight at 37°C in TSB (Difco, Detroit, Mich.). The concentrations were approximated by using a Spectronic 20 (Bausch & Lomb, Rochester, N.Y.) and confirmed by quantitative colony counting. Bacteria were killed by being heated for 1 h at 70°C. Half of the bacteria were labeled with fluorescein isothiocyanate (FITC) for the phagocytosis assay, and the other half

* Corresponding author. Mailing address: Department of Surgery, University of Louisville, Louisville, KY 40292. Phone: (502) 852-5442. Fax: (502) 852-8915.

remained unlabeled for measurement of superoxide production. Fluorescent labeling of bacteria was performed as previously described (4).

The bacteria were washed with phosphate-buffered saline (PBS), resuspended in PBS to a concentration of 10^9/ml, and labeled by incubation with 0.1 mg of FITC isomer 1 (Sigma Chemical Co., St. Louis, Mo.) per ml in 0.1 M $NaHCO_3$, pH 9.0, for 120 min at 25°C. The bacteria were then washed with PBS and centrifugated at 11,000 × *g* for 20 min to remove the unbound fluorochrome. The bacteria were resuspended to 10^9/ml of PBS, aliquoted, and stored frozen at −70°C until used. Aliquots were thawed just before use.

Serum. Serum was isolated from 10 healthy volunteers and pooled. All studies adhered to the guidelines of the U.S. Department of Health and Human Services and the University of Louisville Human Studies Committee. Whole blood was collected in serum separator tube gel and clot activator Vacutainers (Becton Dickinson, Rutherford, N.J.), refrigerated for 1 h at 4°C, and centrifuged at 700 × *g* for 30 min at 4°C.

Isolation of human neutrophils. Human PMNs were isolated from four healthy volunteers by density gradient centrifugation (2). Briefly, whole blood was collected in acid citrate dextrose-containing Vacutainers and layered onto histopaque-1077 and histopaque-1119 (Sigma Chemical Co.). The gradient was centrifuged at 700 × *g* for 30 min at room temperature. The upper mononuclear layer was removed and discarded, the PMNs were collected from the second layer, and erythrocytes remained in the bottom pellet. Remaining erythrocytes within the PMN layer were removed by hypotonic lysis (ammonium chloride at 150 mM, potassium bicarbonate at 12 mM, EDTA at 0.1 mM) for 12 min at room temperature. PMNs were washed two or three times with Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution and pelleted by centrifugation at 250 × *g* for 15 min at room temperature. PMNs were resuspended in PBS at a concentration of 10^7/ml.

Phagocytosis assay. The phagocytosis assay (4) was performed in polypropylene tubes with a final volume of 0.5 ml/tube. One million PMNs were added to each tube for each time period to be studied. The stock bacteria (10^9/ml) was diluted with PBS to 4×10^7 bacteria/ml, and 100 μl (4×10^6 bacteria) was added to each tube, resulting in a bacterium-to-PMN ratio of 4 to 1. After addition of 50 μl of serum, PBS was added to each tube to a final volume of 0.5 ml. Parafilm was placed over the tubes, and they were incubated in a shaking water bath at 37°C with continuous agitation for 30 s and 2, 5, 10, and 30 min, respectively. Samples were removed at the end of each time period, placed on ice, centrifuged in a microcentrifuge for 1.5 min at 1,000 × *g*, and washed twice with ice-cold PBS to remove excess FITC-labeled bacteria. The PMNs were resuspended in 0.5 ml of ice-cold PBS with 5% fetal calf serum and 5 mM glucose. To quench the fluorescence of adherent bacteria, ethidium bromide (EB) was added to a final concentration of 50 μg/ml. The addition of EB was done after the first acquisition by FACScan (Becton Dickinson Immunocytometry Systems, San Jose, Calif.) and 10 min before the second acquisition. Quenching with EB reduced the FITC fluorescence of adherent bacteria by excitation energy transfer (11, 30), and unquenched FITC fluorescence from internalized bacteria was used to assess ingestion and the phagocytic index (9).

Oxidative-burst response assay. The production of superoxide during oxidative burst was measured by the conversion of hydroethidine (HE; Sigma Chemical Co.) to EB with flow cytometry as described by Rothe and Valet (24). HE (50 μl, 0.5 μg) was added 15 min before the bacteria. The phagocytosis assay that determined ROI production was prepared as described in the section above and used unlabeled bacteria instead of FITC-labeled bacteria. FACScan analysis determined EB fluorescence (FL2-H) in PMNs.

Flow cytometry. A FACScan emitting an argon laser beam at 488 nm (Becton Dickinson Immunocytometry Systems) was used to detect both FITC and EB fluorescence. Fluorescence values (FL1-H, green; FL2-H, red) were collected after gating granulocytes in the combination of forward light scatter and perpendicular light scatter (region 1). The compensation (FL1-H minus FL2-H) was set at 1.0 for the phagocytosis assay and at 29 for the ROI assay. A total of 5,000 PMNs were analyzed per tube, and acquired data were processed with the Cellquest version 1.0 software (Becton Dickinson Immunocytometry Systems). The percentage of fluorescent PMNs, the mean fluorescence intensity (MFI) of all PMNs, and the MFI of FL1-H-positive PMNs were determined in each case. The events were acquired in a logarithmic mode for FL1-H and FL2-H. Gating region 2 was determined in the contour plots and included all cells with an FL2-H lower than 4,000 (see Fig. 1). This allowed us to exclude dead and highly permeable PMN with high red fluorescence (>4,000) when samples were analyzed after the addition of EB. The threshold level was set at 45 for FL1-H and FL2-H.

HIC. To determine differences in hydrophobicity between *S. epidermidis* RP62A and RP62A-NA, modified hydrophobic-interaction chromatography (HIC) was performed as previously described (27, 29). Briefly, Pasteur pipettes were used as columns. The tip of each pipette was plugged with glass wool, and the pipette was filled with 1 ml of octyl-Sepharose gel (Sigma Chemical Co.). The columns were equilibrated with 10 ml of starting buffer [1 M $(NH_4)_2SO_4$ (PBA) in 0.001 M phosphate buffer, pH 6.8] to remove fine particles, and ethanol was added to the gel as a preservative. The test bacteria (10^9 in 250 μl of starting buffer) was placed on top of the column and eluted in 1-ml increments. Each eluate consisted in 1 ml of PBA in decreasing concentrations of 1 to 0.01 M. Finally, distilled water and 95% ethanol were used. All eluents except H_2O and ethanol were at 0.01 M with respect to phosphate and had a pH of 6.8. The eluates were collected in separate tubes after their passage through the column, and the optical density (OD) was measured at 410 nm with an MR4000 enzyme-linked immunosorbent assay reader (Dynatech Laboratories, Inc., Chantilly, Va.). The OD at 410 nm was converted to the total number of bacteria as determined by standard curves for each bacterium.

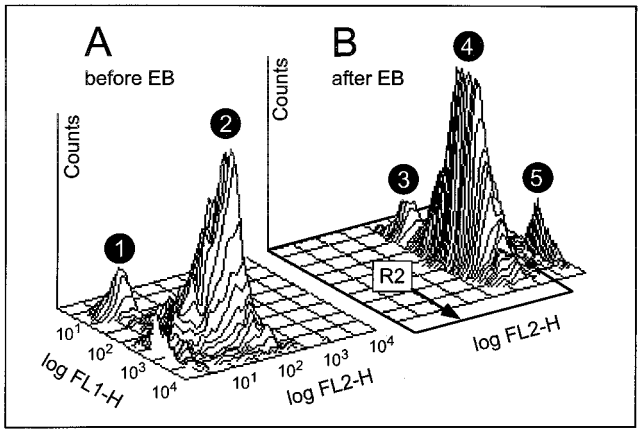

FIG. 1. Representative three-dimensional histogram of PMNs exposed for 10 min to *S. epidermidis* RP62A-NA and analyzed by flow cytometry. (A) Fluorescence values before addition of EB. (B) Fluorescence values after addition of EB and quenching of adherent, extracellular bacteria. Gating region R2 was set to an FL2-H fluorescence value of 4,000 and excluded dead and highly permeable PMNs. The five peaks indicate the following different PMN populations: 1, PMNs without phagocytosis of bacteria; 2, PMNs with adherent and ingested bacteria; 3, PMNs without phagocytosis of bacteria or with adherent bacteria only; 4, PMNs with ingested bacteria only; 5, dead PMNs with high permeability for EB.

Microscopy. To correlate data from the FACScan with light microscopy, we used a fluorescence microscope (Axioscope; Zeiss). We analyzed 20 experiments by counting the number of green (ingested) bacteria and red (adherent) bacteria in 50 PMNs of each experiment.

Data analysis. Ingestion was measured by the presence of green fluorescence (FL1-H value) after addition of EB (and quenching of adherent bacteria), and adherence was determined by calculating the difference in green fluorescence after quenching with EB from that in unquenched samples. The MFI of PMNs with phagocytosed *S. epidermidis* (FL1-H-positive PMNs) was used to determine the phagocytosis index, in analogy to the classical phagocytic index that assesses the average number of bacteria per phagocyte. The phagocytosis index is the FL1-H value after quenching with EB in FL1-H-positive PMNs. The adherence index is the FL1-H value before quenching with EB minus the FL1-H value after quenching with EB in FL1-H-positive PMNs.

Statistical analysis. Analysis of variance (ANOVA) was performed to compare the data of phagocytosis and ROI production between *S. epidermidis* RP62A and RP62A-NA at each time period. A P value of <0.05 was considered significant.

RESULTS

PMNs. We chose to study isolated PMNs to obtain homogeneous cell populations at equal concentrations. The viability of PMNs after isolation was 96 to 99% as determined by trypan blue exclusion. The cells' death resulted in increased permeability to EB and was reflected in the percentage of PMNs with a very high level of red fluorescence. Defining gating region 2 in flow cytometry excluded all PMNs with a red fluorescence intensity of over 4,000 (Fig. 1) and allowed us to analyze viable PMNs. The percentage of cells excluded by this method was 0.2 to 3%, correlating closely with the viability results after staining with trypan blue.

Bacteria. The staining of bacteria with FITC yielded different levels of fluorescence in the two strains of *S. epidermidis*. The MFI of bacteria was reflected by FL1-H values of 856 for *S. epidermidis* RP62A and 1,347 for *S. epidermidis* RP62A-NA. Adding EB to the FITC-labeled bacteria reduced FL1-H to 86 for RP62A and reduced FL1-H to 81 for RP62A-NA, indicat-

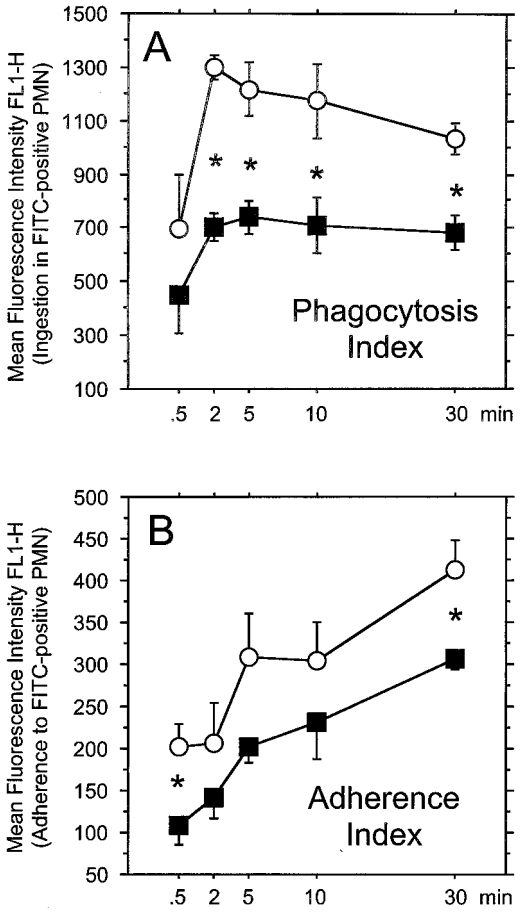

FIG. 2. Fluorescence intensities of FL1-H-positive PMNs, i.e. PMNs that phagocytosed FITC-labeled *S. epidermidis*. (A) Comparison of the phagocytosis index of slime-producing *S. epidermidis* RP62A (○) with that of non-slime-producing *S. epidermidis* RP62A-NA (■). The data reflect the fluorescence intensity in FL1-H-positive PMNs after quenching of extracellular FITC-labeled bacteria with EB. (B) Comparison of the adherence index of slime-producing *S. epidermidis* RP62A (○) with that of non-slime-producing *S. epidermidis* RP62A-NA (■). The data reflect the calculated difference in the fluorescence of FL1-H positive PMNs before and after quenching of adherent FITC-labeled bacteria with EB. The values are means ± the standard error of the mean. Significance of differences (*, $P < 0.05$) was assessed by ANOVA.

ing effective quenching of the FITC fluorochrome of non-ingested bacteria. In preliminary studies, we tried to label live and replicating bacteria with FITC but those experiments were not successful. We were therefore not able to study live bacteria.

Phagocytosis. The correlation between light microscopy and flow cytometry for the percentage of cells that phagocytosed *S. epidermidis* was $R^2 = 0.903$, which indicates accurate FACScan measurements. The number of ingested bacteria and the FL1-H value in FL1-H positive PMNs correlated with an R^2 value of 0.796.

Slime-producing *S. epidermidis* RP62A showed a consistently higher phagocytosis index than the non-slime-producing variant RP62A-NA over the entire experiment (Fig. 2A), and this difference reached significance ($P < 0.05$) at 2, 5, 10, and 30 min. Also, *S. epidermidis* RP62A showed a consistently higher bacterial adherence index, a difference which was significant at 30 s and 30 min (Fig. 2B). Both bacteria showed similar kinetics in adherence to, and phagocytosis by, PMNs. After 30 s, 62% ± 10% and 63% ± 11% of the PMNs phagocytosed *S. epidermidis* RP62A and RP62A-NA, respectively. The percentage of PMNs that phagocytosed bacteria reached a plateau at 2 min (85% ± 1% for RP62A and 84% ± 1% for RP62A-NA). By 30 min, 90% ± 1% of the PMNs phagocytosed RP62A and 91% ± 1% of the PMNs phagocytosed RP62A-NA. After 2 min, the percentages of PMNs with only adherent bacteria were 2% ± 2% and 3.5% ± 1% for RP62A and RP62A-NA, respectively. A plateau was reached after 5 min, at which 7% ± 1% of the PMNs had either RP62A or RP62A-NA adherent.

Because of the different initial FL1-H values of the two bacteria, the FL1-H results of the phagocytosis assay were adjusted by a factor of 1.17 for RP62A and by a factor or 0.74 for RP62A-NA. This allowed us to compare theoretically identical FL1-H values of 1,000 for both bacteria. To prove the accuracy of this transformation, we performed a separate phagocytosis assay with two donors by using two different initial FL1-H values for RP62A: 2,042 for RP62A 1 and 1,715 for RP62A 2. Apart from the MFI, there were no differences. Both bacteria were labeled from the same batch, treated identically, and used in the same assay. The results showed a mean difference in FL1-H values of 118.8% between RP62A 1 and RP62A 2 for ingestion, correlating closely with the difference of 119.1% between the FL1-H values of the bacteria themselves. Another strong indication of a relevant difference was the consistently higher FL1-H value after phagocytosis of RP62A (even with a lower initial MFI) than RP62A-NA when each donor was analyzed separately.

ROI production. While the phagocytic index can assess the ingestion of bacteria, it cannot determine subsequent steps in microbial killing, such as the production of ROI. To address this question, we incubated PMNs from the same experiment with unlabeled bacteria and measured the generation of superoxide by the conversion of nonfluorescent HE to fluorescent EB. The increase in the percentage of PMNs producing superoxide after phagocytosis of RP62A-NA was delayed by 2 min compared to that of RP62A (Fig. 3A), and the difference was significant at 2 min. Also, the amount of superoxide produced by PMNs phagocytosing RP62A was constantly higher than that produced by PMNs phagocytosing non-slime-producing *S. epidermidis* (Fig. 3B), and the difference was significant at 2 min. These results are consistent with the increased phagocytic index of RP62A (Fig. 2A).

HIC. The increase in phagocytosis of *S. epidermidis* RP62A in suspension could be a result of increased hydrophobicity. However, after growth in TSB, heat killing, and FITC labeling, *S. epidermidis* RP62A proved to be less hydrophobic than its non-slime-producing variant, RP62A-NA. *S. epidermidis* RP62A could be washed out of the hydrophobic octyl-Sepharose gel by salt elution (Fig. 4A), whereas RP62A-NA was washed out only by H_2O or ethanol (Fig. 4B). While the surface properties of *S. epidermidis* could be altered by the preparation procedure, we measured the hydrophobicity of *S. epidermidis* before and after heat killing and FITC labeling. The results indicate that neither heat killing nor FITC labeling changed the general pattern of decreased hydrophobicity of RP62A (Fig. 4A) compared with RP62A-NA (Fig. 4B), indicating an intact slime coat during the phagocytosis assay.

DISCUSSION

We report that slime-coated *S. epidermidis* RP62A demonstrates increased phagocytosis, adherence, and ROI production by human PMNs compared with its non-slime-producing phenotypic variant, RP62A-NA, when analyzed in suspension.

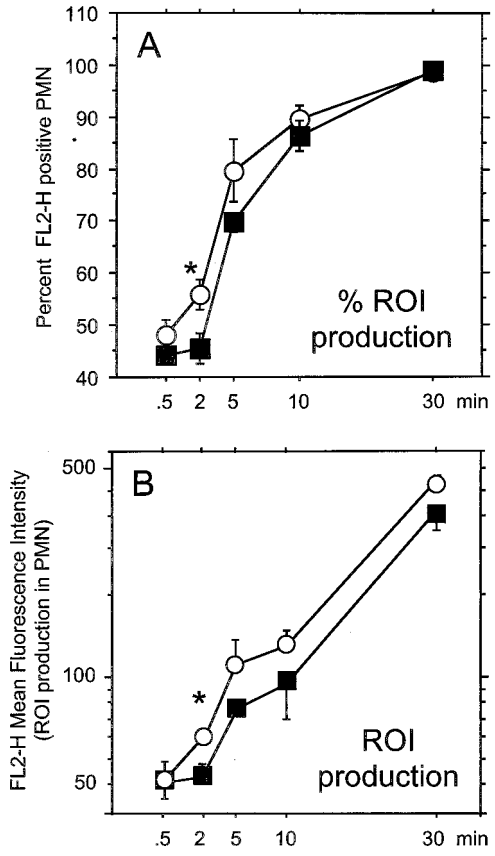

FIG. 3. Production of superoxide by PMNs during oxidative burst and phagocytosis as measured by the percentage of PMNs with FL2-H fluorescence intensity derived from the superoxide-induced conversion of HE to fluorescent EB (A) and mean fluorescence intensity levels (log scale) of slime-producing bacteria (*S. epidermidis* RP62A) (○) and non-slime-producing bacteria (*S. epidermidis* RP62A-NA) (■). The values are means ± the standard error of the mean. Significance of differences (*, $P < 0.05$) was assessed by ANOVA.

This finding was surprising because many reports analyzing slime-producing and non-slime-producing strains of coagulase-negative staphylococci suggest impaired immunity against slime-producing strains. However, most of these studies were performed in phagocytosis models using extracellular slime preparations (10, 12, 23) or bacteria adherent to surfaces (7, 12, 20), mimicking the clinical scenario of bacterial biofilms on implants or foreign bodies. We chose to investigate the phagocytosis of *S. epidermidis* in suspension to mimic the bacteremic phase of *S. epidermidis* dissemination.

Our results are different from those of Kristinsson et al. (14), who studied 43 strains of planktonic *S. epidermidis* for opsonophagocytosis by luminol-dependent chemiluminescence, a by-product of PMN respiratory burst, and by bacterial-killing assays. They found that the presence of extracellular slime (20 strains) had no influence either on the opsonic requirement or on the efficiency of opsonization. Bacterial killing was not different in slime-producing bacteria. Chemiluminescence induced by unopsonized slime-producing *S. epidermidis* was slightly increased; however, there was no difference in chemiluminescence between opsonized slime-producing and opsonized non-slime-producing strains. Thus, the authors demonstrated that there was no increase in phagocytosis in the presence of extracellular slime but, more importantly, also no decrease in phagocytosis. They concluded that extracellular slime had no specific antiopsonic property in vitro. However, the authors did not evaluate slime-producing and non-slime-producing variants of the same strain. Our results, however, show a difference between slime-producing and non-slime-producing bacteria in two variants of the same strain.

Johnson et al. (13) demonstrated that addition of crude slime to PMNs increased *N*-formyl-methionyl-leucyl-phenylalanine-induced superoxide production, assessed by the superoxide dismutase-inhibitable reduction of cytochrome *c*. These observations are supported by our results. The same authors indicated that extracellular slime reduced the superoxide response of PMNs in a surface phagocytosis assay, a finding that

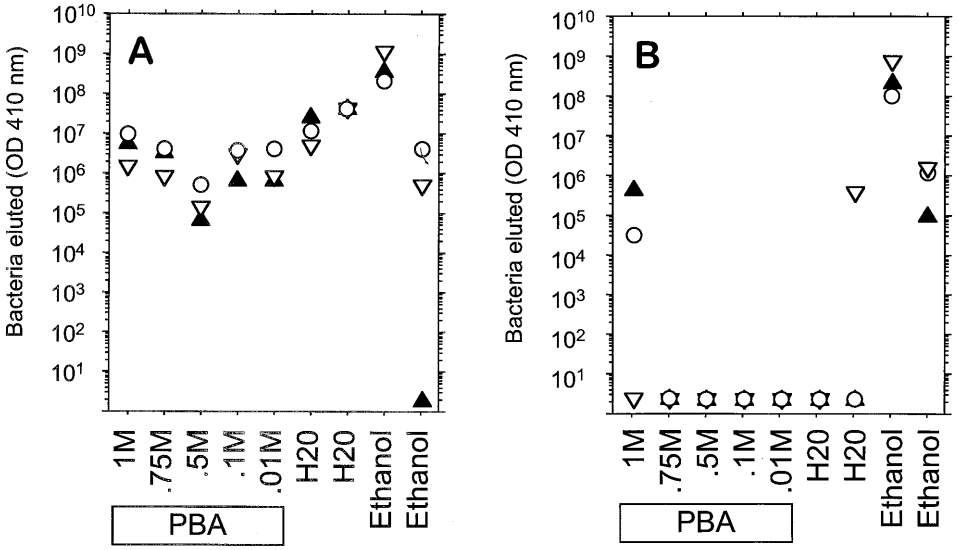

FIG. 4. Hydrophobicity of bacteria at different stages of the preparation procedure for slime-producing *S. epidermidis* RP62A (A) and non-slime-producing *S. epidermidis* RP62A-NA (B). Hydrophobic octyl-Sepharose gel was equilibrated with 1 M PBA in 0.001 M phosphate buffer, and 10^9 bacteria were placed on top of the gel and eluted with 1-ml increments of PBA in decreasing concentrations (1 to 0.01 M) and H_2O. Ethanol was added to remove the remaining bacteria. The OD of the eluted bacteria was read at 410 nm. Symbols: ▲, bacteria grown in tryptic soy broth, ▽, bacteria after heat killing; ○, bacteria after heat killing and staining with FITC. Increased OD after elution with PBA and H_2O demonstrated low hydrophobicity of slime-producing bacteria.

is consistent with the generally accepted theory of the protective properties of slime in bacterial biofilm. These studies add supporting evidence to our postulate that phagocytosis of *S. epidermidis* is dependent on the phagocytosis assay (i.e., planktonic versus surface phagocytosis assays).

Extracellular slime produced by *S. epidermidis* has different activating effects on human PMNs (13) such as increased chemotaxis, an increased early superoxide response to *N*-formyl-methionyl-leucyl-phenylalanine, enhanced adherence to plastic, and increased degranulation of lactoferrin and myeloperoxidase. A possible teleological explanation for this phenomenon might be an aggressive host response to slime-producing bacteria. This is especially important during bacteremia (i.e., in the planktonic phase). Clearance of bacteria is very much impaired when bacteria have established a bacterial biofilm after adherence to material or tissue surfaces, accumulation, and slime production. On the other hand, growth within protected glycocalyx-enclosed biofilms also imposes several limitations on bacterial pathogenicity. Bacteria and toxins are retained within the biofilm matrix and can be neutralized by antibodies or phagocytes of the biofilm surface. It is poorly understood why, after a dormant period between colonization and growth, the bacterial biofilm eventually results in clinically relevant infection (1). Initially, the bacterial biofilm creates no clinically recognizable signs of infection, but these biofilms do represent a nidus of infection when the host defense fails to contain them and planktonic bacteria are disseminated. Conventional therapeutic doses of antibiotics are often effective in killing disseminated bacteria, but the bacteria in the biofilm are not eradicated, which leads to persistence of the nidus. Total eradication of the implant is an important principle of treatment (5). Extensive bacterial biofilms have been found on many implanted devices (1, 8, 26, 31), such as shunts and catheters, vascular grafts, heart valves, endotracheal tubes, and prosthetic hip joints.

Yasuda et al. (36) have investigated the interaction between human PMNs and *Escherichia coli* released from an in vitro bacterial biofilm model that was formed on surfaces of prepared cotton threads. The sensitivity of these slime-bearing bacteria to phagocytosis was not different from that of normal bacteria grown in artificial medium. However, these results cannot necessarily be compared to those obtained with the *S. epidermidis* variants RP62A and RP62A-NA because *E. coli* has a capsule and does not produce slime.

The use of flow cytometry is an improvement over time-consuming and subjective methods of manually counting bacteria, and various protocols have been published to measure phagocytosis (15, 18, 28, 34). The problem of differentiating ingested from adherent bacteria could be resolved by using EB to quench adherent, extracellular bacteria (9). The disadvantage of flow cytometry compared with microscopy is the lack of assessment of the exact number of ingested bacteria in each PMN. However, measurements of the percentage of PMNs with phagocytosed bacteria and the number of fluorescence intensity units reflecting the number of ingested bacteria are accurate. The advantage of flow cytometry over microscopy is the ability to analyze several thousand cells within seconds and to obtain objective and reproducible results. In addition, the use of EB and determination of gating area R2 allow the exclusion of dead cells due to their intense red fluorescence (Fig. 1).

Hydrophobicity of bacteria has generally been correlated with increased phagocytosis by PMNs (32), with enhanced virulence (25), and with increased attachment to the surface of implanted devices (5). Our results showed enhanced phagocytosis of *S. epidermidis* RP62A, despite its lower hydrophobicity. Therefore, mechanisms other than hydrophobicity might be responsible for this phenomenon. One possibility is increased opsonization of RP62A compared with RP62A-NA, which is a hypothesis that could not be confirmed by Kristinsson et al. (14). The other possibility is that other nonopsonic mechanisms are responsible, such as lectinophagocytosis, which is based on recognition between surface lectins on one cell and carbohydrates on the opposing cell or other mechanisms requiring adhesion reactions (17). Possible ligands on the bacterial side are proteins such as polysaccharide adhesin, a unique capsular adhesin produced by most strains of *S. epidermidis*, including RP62A and RP62A-NA. Muller et al. (16) demonstrated that *S. epidermidis* polysaccharide adhesin was closely associated with slime production.

In summary, we have shown that planktonic, slime-producing *S. epidermidis* RP62A induces increased phagocytosis by PMNs and generates increased production of ROI during oxidative burst compared with its non-slime-producing phenotypic variant RP62A-NA. We also demonstrated that the slime coat of *S. epidermidis* reduced the bacteria's hydrophobicity, but not by a sufficient amount to decrease phagocytosis and ROI production. The increased phagocytosis of slime-producing *S. epidermidis* RP62A in suspension is potentially important during bacteremia to reduce adhesion, accumulation, and the genesis of a bacterial biofilm on implants or tissue surfaces.

ACKNOWLEDGMENTS

We thank A. J. Roll for excellent technical assistance with flow cytometry and J. C. Peyton for reviewing the manuscript.

This work was supported in part by the John W. and Caroline Price Trust, the Alliant Community Trust, and the Mary and Mason Rudd Endowment Fund of Jewish Hospital (Louisville, Ky.).

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