The interaction of Staphylococcus epidermidis slime with human neutrophils (PMN) was examined by using isolated slime and allowing bacteria to elaborate slime in vitro. Staphylococcus epidermidis slime was found to contain chemotactic factor(s) for PMN. Subsequent studies have shown that PMN activated by slime from Staphylococcus epidermidis adhered to plastic. This interaction has been recognized as an example of a specific, slime-dependent adherence to plastic. It was also shown that slime from Staphylococcus epidermidis could stimulate PMN to produce a myeloperoxidase-containing granule that was released from the PMN. This granule release was inhibited by slime from Staphylococcus epidermidis but not by slime from Staphylococcus aureus. These results suggest that the slime interaction with PMN may be promoted by the bacterial surface.
4°C, concentrated by filtration, and lyophilized. This bacteria-free slime was dissolved in Hanks balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.) with 0.1% gelatin (GHBSS) for use in the experiments.

Preparation of bacterial cells. S. epidermidis KH11 and V2, another slime-producing strain, were used for phagocytosis experiments. Staphylococcus aureus Cowan 1 was used as the comparison bacteria for the phagocytosis experiments. S. epidermidis strains were grown overnight in nutrient broth supplemented with 3% Casamino Acids, and 1% dextrose with tritiated adenine (specific activity, 40 Ci/mmole; Research Products International Corp., Mount Prospect, Ill.) (4 μCi/ml) was added for radiolabeling of the bacteria. S. aureus were grown in Mueller-Hinton broth (Difco) with tritiated adenine (4 μCi/ml). The bacteria were harvested by centrifugation at 2,000 × g for 10 min, washed twice in phosphate-buffered saline (PBS), and resuspended at 2 × 10⁷/ml in PBS by using a spectrophotometric method. For some experiments, S. epidermidis and S. aureus were allowed to grow in their respective media in 24-well plastic plates (Costar, Cambridge, Mass.) as described in detail for the surface phagocytosis assay.

Granulocyte preparation. Polymorphonuclear neutrophils (PMNs) were prepared for chemotaxis studies by the method of Ferrante and Thong (17). Heparinized blood from healthy volunteers was layered on high-density (1.141 g/ml) Ficoll-Hypaque (Mono-Poly Resolving Media; Flow Laboratories, Inc., McLean, Va.) and centrifuged at 300 × g for 45 min. The PMN layer was removed and washed twice with GHBSS, and the PMNs were resuspended at appropriate concentrations.

A modified method of Boyum (8) was used to prepare the PMNs for phagocytosis and degranulation studies with dextran sedimentation of the leukocyte-enriched plasma layer at 200 × g for 10 min. Contaminating erythrocytes were removed by hypotonic lysis with distilled water for 20 s followed by the restoration of isotonicity by the addition of an equal amount of 2 × Hanks balanced salt solution (2 × HBSS). The leukocytes and lysed erythrocytes were layered on Ficoll-Hypaque (LSM; Litton Bionetics, Kensington, Md.) and centrifuged at 300 × g for 30 min. The PMN pellet obtained was washed twice as above and resuspended in GHBSS at appropriate concentrations.

Cells obtained by both methods were greater than 94% PMNs, and viability was greater than 96% as assessed by trypan blue dye exclusion.

Chemotaxis. The underagarose technique of Nelson et al. (41) with the modifications of Chenoweth et al. (10) was used to measure chemotaxis. Each plastic petri dish was pretreated with 1 ml of 0.5% gelatin for 1 h at room temperature and then gently rinsed once with distilled water. After drying, each dish was filled with 6 ml of molten agarose mixture containing 1% agarose and 0.5% gelatin in minimal essential medium buffered to pH 7.4 with 50 mM HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid) buffer (Sigma Chemical Co., St. Louis, Mo.). Rows of three wells 3 mm apart were cut in the solidified mixture immediately before application of chemoattractants and PMNs. Slime was used as an attractant in some experiments and in other experiments slime was incubated with PMNs, as described below, and then the chemotactic responsiveness to the known chemotaxins, zymosan-activated serum (ZAS) and 10⁻⁸ M n-formyl-methionyl-leucyl-phenylalanine (FMLP; Sigma), was assayed. GHBSS was used as the control attractant for spontaneous migration. The PMNs were applied to the plates at 2.5 × 10⁷ to 5 × 10⁷/ml after the application of the attractants. The migration of the PMNs was measured after exposure (2 to 2.5 h) to the attractants at 37°C in a humidified 5% CO₂ atmosphere followed by fixation overnight at 4°C with 2.5% glutaraldehyde. The plates were stained with Wright stain and examined under a projecting microscope at ×40 with a scaled grid. Migration was determined by the leading-edge method as the farthest distance migrated by at least three cells. Values for directed migration are expressed as the chemotactic differential, the directed migration minus random migration. For all experiments with incubated PMNs, migration was assessed as a percentage of the migration of the control incubated PMNs. All chemotaxis experiments were performed in triplicate or quadruplicate.

Incubation. PMNs at 5 × 10⁹/ml were incubated in the presence of 10% heat-inactivated pooled human sera either with various concentrations of crude slime in GHBSS or with GHBSS alone at 37°C in a humidified 5% CO₂ atmosphere for 15 min. The cells were then centrifuged at 200 × g for 10 min. The supernatant was gently removed and assayed for lactate dehydrogenase (LDH) as previously described (61), and the cells were washed twice with GHBSS. The PMNs were finally suspended in GHBSS at 2.5 × 10⁹ to 5 × 10⁹/ml for use in the chemotaxis assay described above. Cell viability was also assessed by trypan blue dye exclusion after incubation.

Chemotactic. For preparation of ZAS, 1 volume of activated zymosan (Sigma) at 50 mg/ml in PBS (pH 7.4) was added to 3 volumes of pooled human sera. The mixture was incubated rotating for 30 min at 37°C. Zymosan was then removed by centrifugation at 1,500 × g for 10 min. The ZAS was then heated for 30 min at 56°C, aliquoted, and stored at −70°C. FMLP was dissolved at 10⁻³ M in dimethyl sulfoxide (Sigma), diluted to 10⁻³ M with GHBSS, aliquoted, and frozen at −70°C. A final dilution to 10⁻⁸ M with GHBSS was performed immediately before use.

Degranulation. PMNs at a final concentration of 3 × 10⁶/ml were exposed to slime or appropriate buffer control after a 5-min treatment of cells with or without cytochalasin b (Sigma) at 37°C. Various concentrations of slime were compared to opsonized zymosan and FMLP. The final incubation volume was 1 ml. Degranulation was assessed at zero time and after 30 min at 37°C. The cells were centrifuged at 300 × g for 10 min at 4°C, and the supernatants were aspirated gently and kept at 4°C for the granule content assays or at room temperature for the LDH assay. The specific granule content release was assessed by lactoferrin (LF) release, and azurophil granule release was assessed by myeloperoxidase (MPO) release. LF was measured in an enzyme-linked immunosorbent assay (27), and MPO was measured by orthodianisidine colorimetric assay measured at 450 nm (26). LF and MPO were expressed as the percentage of the total available from the PMN suspension determined after lysis with PBS containing 0.1% Triton-X-100 (Sigma) and 0.5 mM NaCl. The degranulation experiments were performed in duplicate. Viability after degranulation was assessed by trypan blue dye exclusion and LDH release.

Phagocytosis. Phagocytosis was measured by the recently described surface phagocytosis technique (30) modified to allow for bacterial growth on the plate at 37°C for 18 h. Briefly, 1 ml of radiolabeled bacteria in PBS was added to the wells of 24-well plastic plates (Costar) and allowed to adhere to the plate at 37°C for 2 h. Alternatively, the same strains of S. epidermidis, KH11 and V2, and S. aureus were added to the wells with 1 ml of appropriate radiolabeled medium and incubated for 18 h at 37°C. For some experi-
ments, S. epidermidis KH11 was incubated in the wells in radiolabeled nutrient broth without supplementation to allow growth without promoting slime production by the organism (34). Preliminary experiments measuring the number of CFU removed from the appropriate wells after adherence or incubation and determining the specific radiolabeling per CFU of the bacteria grown under the different conditions assured that equal numbers of adherent or grown bacteria after incubation were present for uptake by PMNs. This information was used to determine the ratio of bacteria to PMNs and to check for equal numbers of bacteria for all experiments. The supernatant and nonadherent bacteria were removed by gentle aspiration. To initiate phagocytosis, 0.5 ml of PMNs at \(2.5 \times 10^6\) cells per ml was added to each well at a 10:1 to 50:1 bacteria to PMNs ratio, with or without 10% pooled human sera added for opsonization. The plates were then incubated (stationary) at 37°C for 15 or 60 min. After incubation, the supernatant was aspirated and saved. The adherent bacteria and PMNs were removed from the plates by the addition of 1 ml of 0.87% NaCl containing 0.5% trypsin and 0.2% EDTA and a 15-min incubation at 37°C. The contents of the wells were then aspirated and added to the appropriate supernatants. The PMNs were separated from the nonphagocytized bacteria by differential centrifugation and washing of PMN pellets with PBS. The supernatants containing nonphagocytized bacteria were saved, and the bacteria were sedimented at \(2,000 \times g\) for 10 min. Aquasol (3 ml; New England Nuclear Corp., Boston, Mass.) was added to both the washed PMN pellet and the final bacterial pellet in polypropylene vials (Biovial; Beckman Instruments, Inc., Fullerton, Calif.). The radioactivity of the vials was then measured on a scintillation counter. Percent phagocytosis was calculated as follows: % phagocytosis = \(A/(A + B) \times 100\), where \(A\) is the radioactivity associated with the PMNs, and \(B\) is the radioactivity associated with the bacterial pellet. Identical preliminary experiments were performed with bacteria without radiolabeling. Microscopic evaluation after PMN application and incubation (30), examining at least 10 PMN for each treatment, revealed that 10% or less of the bacteria associated with PMNs appeared to be adhered to the surface of the PMNs.

Statistics. All data are expressed as means ± standard error of the mean of results of at least three separate experiments. A two-tailed \(t\)-test was used for statistical analysis. \(P\) values >0.05 were not considered significant. Statistical results for chemotaxis experiments are reported for the percentage of the control migration.

RESULTS

Chemotaxis. S. epidermidis slime was found to contain a chemotaxin for human PMNs. At concentrations greater than 1 mg/ml, a significant chemoattractant effect was observed. From 0.1 to 50 mg/ml, the migration differential increased from 0.05 to 4.2 cm. The chemotactic response was dose related when the migration differential was plotted against the log of the slime concentration (\(r = 0.943\); Fig. 1). This effect was not altered by heating at 56°C for 30 min, and medium used for enhanced slime production was not itself chemotactic (results not shown).

Preincubation of PMNs with slime inhibited subsequent chemotactic responsiveness of the cells to the known chemoattractants, ZAS and FMLP. When cells were incubated with increasing concentrations of S. epidermidis slime, the inhibition of PMN response to ZAS ranged from 24.1 ± 11.2% at 10 \(\mu\)g/ml, which was not significantly different, to 67.5 ± 6.8% at 250 \(\mu\)g/ml (\(P < 0.001\)) when compared with the migration of the control incubated cells and expressed as the percentage of the control migration (Fig. 2). A similar but less marked decrease in chemotactic response to FMLP was also demonstrated by PMNs preincubated with S. epidermi-
**Phagocytosis.** *S. epidermidis* which was incubated for 18 h in medium which promoted slime production on the plastic plates to allow in situ slime production were not phagocytized as readily by PMNs as was *S. epidermidis* adhered to the plate for 2 h. The uptake of unopsonized 2-h adherent *S. epidermidis* KH11 and V2 was 29 ± 1.6% and 63 ± 2.4%, respectively, but was only 11 ± 0.8% and 31 ± 4.0% of that of *S. epidermidis* grown on the plate for 18 h (*P* < 0.001 for both strains) (Fig. 3). Parallel differences were observed with *S. epidermidis* opsonized with 10% PHS. The 15-min uptake of opsonized *S. epidermidis* KH11 and V2 adhered for 2 h was 57 ± 4.0% and 83 ± 1.4%, respectively, but the uptake of *S. epidermidis* incubated for 18 h and opsonized was only 21 ± 1.8% and 50 ± 3.6% (*P* < 0.001 for both strains). At 60 min, the uptake of unopsonized *S. epidermidis* KH11 and V2 was 49 ± 2.9% and 80 ± 1.8%, respectively, for 2-h-adherent bacteria compared with 16 ± 2.7% and 60 ± 1.7% for bacteria incubated on the plate (*P* < 0.001). Similarly, the 60-min uptake of opsonized bacteria was 62 ± 3.5% and 88 ± 1.1% for 2-h-adherent bacteria versus 23 ± 2.6% and 63 ± 2.3% for bacteria incubated for 18 h (*P* < 0.001). Although opsonization increased the PMN uptake of *S. epidermidis* incubated in situ for 18 h, it did not correct the diminished uptake relative to washed adherent bacteria. *S. epidermidis* KH11 which was incubated on the plastic plates in medium which did not promote slime production was more readily phagocytized than bacteria grown in the slime-promoting, supplemented medium (Fig. 4). The 15-min uptake of these bacteria without or with opsonization was 18 ± 0.6% and 51 ± 2.2%, respectively, and the 60-minute uptake was 22 ± 2.4% and 58 ± 4.2%. The interference with PMN phagocytosis of *S. epidermidis* allowed to grow on the plate and produce slime could not be demonstrated with the control organism *S. aureus* Cowan 1. There was no difference at either 15 or 60 min in the uptake of *S. aureus* incubated in situ for 18 h (Fig. 3).

**Degranulation.**57.000000000000004% PMN degranulation in response to exo-

**FIG. 3.** PMN surface phagocytosis after 15 or 60 min of radiolabeled *S. epidermidis* V2 (A), KH11 (B), or *S. aureus* (C) adhered to plastic wells for 2 h or incubated in broth in plastic wells for 18 h. The uptake of bacteria by PMNs is presented as the percentage of the total adherent bacteria in the wells. The uptake is measured with or without opsonization of the respective bacteria. Each point is the mean ± the standard error of at least three experiments. *P* values compare the uptake of 2-h-adherent bacteria with the uptake of bacteria incubated for 18 h.

**FIG. 4.** Surface phagocytosis after 15 or 60 min of *S. epidermidis* KH11 incubated in plastic wells for 18 h in 1H-labeled nutrient broth, with or without supplementation to promote slime production. The uptake of bacteria by PMNs is presented as the percentage of the total adherent bacteria in the wells. Each point is the mean ± the standard error of at least three experiments. *P* values compare the uptake of bacteria grown in the supplemented medium with the uptake of bacteria incubated in medium without supplementation. NS, No significant difference.

*dis* slime. The inhibition found at 50 μg/ml (27 ± 8.8%; *P* < 0.02) did not increase with incubation at higher slime concentrations (Fig. 2). Since the comparison of chemotaxis after incubation by either the chemotactic differential or the percentage of the control migration resulted in similar analysis, only the percentage of the control migration is presented. The random migration of PMN was not decreased by the incubations at any of the slime concentrations examined. PMN viability as measured by LDH release (<5%) and by trypan blue dye exclusion (>95%) was not affected by incubation with *S. epidermidis* slime.
sue to concentrations of slime which were found to inhibit chemotactic responsiveness was monitored to further investigate the interaction of this substance with granulocytes. There was little release of MPO from PMNs incubated with *S. epidermidis* slime. Only 2.9 to 3.8% of total cellular MPO was released by untreated PMNs during incubation in slime at concentrations of 10 to 250 μg/ml (Fig. 5). Pretreatment of PMNs with cytochalasin b (5 μg/ml) slightly enhanced MPO release (10 ± 2.1%) at 250 μg of slime per ml (*P < 0.01*). In contrast, *S. epidermidis* slime stimulated LF release from untreated PMNs (13.5 ± 2.9%), but only at the highest concentration of slime examined (250 μg/ml) (Fig. 6). Pretreatment with cytochalasin b enhanced LF release at lower concentrations of slime (7.7 ± 1.9% at 50 μg/ml to 33.8 ± 8.8% at 250 μg/ml [*P < 0.01*]). These findings suggest that *S. epidermidis* slime has a greater effect on the release of specific granule contents than on azurophil granule contents.

**DISCUSSION**

The persistent nature of foreign-body infections with *S. epidermidis* despite apparently adequate antibiotic therapy in normal hosts led us and others to postulate that the extracellular slime produced by these bacteria may sequester the organism from the action of antibiotics and may be able to inhibit host defenses (3, 13, 44, 45). We previously showed that *S. epidermidis* slime interferes with the human cellular immune response by interfering with the lymphoproliferative response to mitogens (23).

We now report that *S. epidermidis* slime alters PMN functions, which may potentially impair the inflammatory response and contribute to the persistence of the bacteria.

PMN responsiveness to chemotactic stimuli was inhibited when cells were preincubated with slime. The mechanism of this interaction is unknown; however, the decreased response to FMLP after slime incubation suggests that the cells may have adapted with increased numbers of FMLP receptors of altered affinity on the surface of the cell (52). This would be consistent with the predominantly specific granule degranulation, since the intracellular pool of FMLP receptors resides in the specific granules (19). The inhibition of response to ZAS may be similarly mediated by changes in the number or affinity or both of the C5a receptors on the surface of the PMNs in response to slime incubation, although the greater inhibition suggests that another interaction may occur at this receptor. Some but not all chemottractants, such as that associated with crude slime, were found to deplete PMNs, with subsequent diminished chemotactic responsiveness (40, 54). Further investigations of the interactions of this material with these chemotactic receptors are necessary to define the mechanism of inhibition of chemotaxis found with crude slime.

Phagocytosis of *S. epidermidis* adherent to a surface is an assay which may model plastic foreign-body infections (30). In this system in which *S. epidermidis* are allowed to adhere to and grow on the surface by incubation for 18 h in a slime-promoting medium, there is a decreased uptake by PMNs compared with the uptake of washed bacteria allowed to adhere for 2 h. This was shown for two strains of *S. epidermidis*, KH11 and V2, although differences in uptake of the two strains were also apparent. Uptake of KH11 grown

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**FIG. 5.** PMN MPO release during a 30-min incubation with various concentrations of crude *S. epidermidis* slime with or without cytochalasin b (5 μg/ml) pretreatment. MPO release is expressed as a percentage of the total PMN MPO (*, *P < 0.01* for PMNs incubated in crude slime compared with GHBSS-incubated PMNs). Each point is the mean ± the standard error of at least three separate experiments.

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**FIG. 6.** PMN LF release during a 30-min incubation with various concentrations of crude *S. epidermidis* slime with or without cytochalasin b (5 μg/ml) pretreatment. LF release is expressed as a percentage of the total PMN LF (*, *P < 0.05* and **, *P < 0.01* for PMNs incubated in crude slime compared with GHBSS-incubated PMNs). Each point is the mean ± the standard error of at least three separate experiments.
in the medium supplemented to promote slime production (34) was decreased compared with uptake of the same bacteria grown for the same amount of time in medium without supplementation. Previous morphologic studies showed that slime is not present in S. epidermidis incubated for 6 h or less, whereas slime is visible after 12 h of incubation (44, 45). Thus, in two systems, interference with phagocytosis of S. epidermidis appears to be related to the presence or production of slime, although of course other bacterial products may also play a role.

Interference with PMN chemotaxis and phagocytosis may contribute to the survival of S. epidermidis as well as other bacterial species in locations exposed to the immune system. Several bacterial products have the ability to compromise host defenses. Escherichia coli lipopolysaccharide (25), E. coli enterotoxin (5), and supernatants from dental pathogens (57) interfere with the chemotaxis of PMNs. Also M-protein-positive group A streptococci were shown to interfere with the chemotactic response of PMNs by decreasing C5a generation in vitro (63). Capsular polysaccharides are known to be antiphagocytic for many organisms (6, 46), and possibly the slime produced by S. epidermidis may function similarly to protect the bacteria in vivo as we have found in vitro.

Additionally, several products of S. aureus, including alpha toxin, protein A, and peptidoglycan, were found to interfere with phagocytic cell function, including phagocytosis and chemotaxis (39, 50, 60). Although S. epidermidis has not been as closely examined for the presence of similar materials, it may share some of these factors with S. aureus (21, 56).

We found that slime-stimulated degranulation of the PMNs with predominantly specific granule content release. This degranulation may interfere with the function of the cells and is possibly related to the decreased chemotactic responsiveness and to the decreased LF in the cells (7). Degranulation of PMNs may effectively waste cellular products after contact with the slime. This may be overly simplistic in that enhanced functional capacity of PMNs after exposure to chemoattractants and with degranulation was reported, although different functions were examined (19, 58).

The exposure of PMNs to nonphagocytizable surfaces, such as foreign bodies, may be a mechanism contributing to enhanced susceptibility to local infection (66). The response of PMNs encountering S. epidermidis slime may further diminish the ability of these cells to phagocytize and kill S. epidermidis.

In summary, we reported the interaction of S. epidermidis slime, a heterogenous extracellular bacterial product, with human PMNs. This material interferes with several PMN functions, including chemotaxis, degranulation, and phagocytosis and may contribute to the ability of S. epidermidis to persist on infected plastic foreign bodies.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI07054 and HL27355 from the National Institutes of Health. G.P. was supported by the Deutsche Forschungs Gemeinschaft.

We thank Marilyn Wilson and Valerie Knase for their secretarial support.

LITERATURE CITED


