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Interference with Granulocyte Function by *Staphylococcus epidermidis* Slime

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The interaction of *Staphylococcus epidermidis* slime with human neutrophils (PMN) was examined by using isolated slime and allowing bacteria to elaborate slime and other extracellular products in situ. *S. epidermidis* slime was found to contain a chemoattractant. Incubation of PMN with 50 μg or more of slime per ml inhibited subsequent chemotaxis of the PMN to *n*-formyl-methionyl-leucyl-phenylalanine by 27% and to zymosan-activated serum by 44 to 67% with increasing slime concentrations. *S. epidermidis* slime stimulated little degranulation of untreated PMN. After pretreatment of PMN with 5 μg of cytochalasin *b* per ml, slime predominantly induced release of specific granule contents (33.8% lactoferrin release by 250 μg of slime per ml versus 10% myeloperoxidase release by 250 μg of slime per ml). By a surface phagocytosis assay, PMN uptake of radiolabeled *S. epidermidis* which were incubated for 18 h on a plastic surface for slime expression was less than that for *S. epidermidis* adhered to the plastic for 2 h or grown in unsupplemented nutrient broth. These results suggest that *S. epidermidis* slime interaction with PMN may be potentially detrimental to host defense and may contribute to the ability of this organism to persist on surfaces of foreign bodies in the vascular or central nervous system.

Staphylococcus epidermidis is a common and generally innocuous inhabitant of the human skin and mucous membranes. It is also a major opportunistic pathogen of transiently or permanently implanted plastic devices, particularly intravascular catheters (1, 12, 35, 43, 48), cerebrospinal fluid shunts (3, 9, 49, 51, 53), orthopedic devices (28, 42, 64), peritoneal dialysis catheters (22, 24, 47, 59), cardiac pacemakers (11, 31, 36, 45), and prosthetic valves (15, 29, 37, 55). It is an increasingly frequent infecting pathogen in neonates (2, 18, 38) and immunocompromised hosts (4, 16, 33, 62, 65), groups which are frequently hospitalized with intravascular catheters in place for extended lengths of time. Foreign-body infections with *S. epidermidis* are often indolent in presentation (31, 49, 53, 59), although more dramatic presentation has been recognized (4, 48). The first observations of the propensity of *S. epidermidis* to cause foreign-body infections were made on infected ventriculoatrial shunts in children in the 1960s (9, 49). Bayston and Penny (3) reported that many strains of *S. epidermidis* responsible for cerebrospinal fluid shunt infections produced an adherent mucoid growth with the production of an alcian blue staining material, which they postulated was important in the colonization of these shunts.

Recently we and others showed that *S. epidermidis* is able to adhere to and grow on surfaces of intravascular catheters and endocardial pacemaker leads in vitro (13, 32, 43, 45). In the further course of colonization, the staphylococci produce an extracellular slime substance, which is probably the same as the mucoid substance Bayston and Penny described (3, 13, 44, 45). Subsequently the in vitro adherent *S. epidermidis* become encased in a matrix of slime (44, 45). Analogous to these in vitro findings, scanning electron microscopy of intravascular catheters removed from patients with *S. epidermidis* infections demonstrated the bacteria embedded in deposits of an amorphous material (20, 35, 43). In a mouse model of foreign-body infection, Christensen et al. (14) demonstrated increased pathogenicity of slime-producing compared to non-slime-producing *S. epidermidis* strains.

In addition to its apparent role in adherence to plastic materials, *S. epidermidis* slime may also play a pathogenic role in foreign-body infections by interfering with host defenses. We previously reported that *S. epidermidis* slime interferes with cellular immune response by inhibiting the lymphoproliferative response of mononuclear cells to polyclonal stimulators, phytohemagglutinin, and streptococcal blastogen A (23). Preliminary studies also suggest that slime decreases phagocytosis of *S. epidermidis* in vitro (W. Regelmann, E. D. Gray, P. Thomas, and G. Peters, Meet. Soc. Pediatr. Res. 1984 [abstr. no. 1131]). Studies of the effects of *S. epidermidis* slime on granulocyte chemotaxis, surface phagocytosis, and degranulation form the basis of this report.

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MATERIALS AND METHODS

***S. epidermidis* slime preparation.** Slime was produced in vitro as previously described (34) during the growth of *S. epidermidis* KH11, a well-characterized slime producing strain. Briefly, nutrient agar supplemented with 3% Casamino Acids (Difco Laboratories, Detroit, Mich.) and 1% dextrose was used to promote slime production. Agar plates were inoculated with 5 ml of bacteria suspended in saline, approximately 10^8 bacteria per ml, and incubated for 48 h at 37°C. The liquid bacteria-slime mixture was collected and dispersed by vigorous vortexing for 5 min. After centrifugation at 2,000 × *g* for 30 min to remove bacterial cells, the supernatant was dialyzed against distilled water for 48 h at

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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/mmol; 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 2×10^9/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 (1.114 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 2.5×10^7 to 5×10^7/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% CO_2 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 5×10^6/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% CO_2 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 2.5×10^7 to 5×10^7/ml for use in the chemotaxis assay described above. Cell viability was also assessed by trypan blue dye exclusion after incubation.

Chemoattractants. 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^{-5} 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 3×10^6/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 M 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 2.5×10^6 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 × *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.

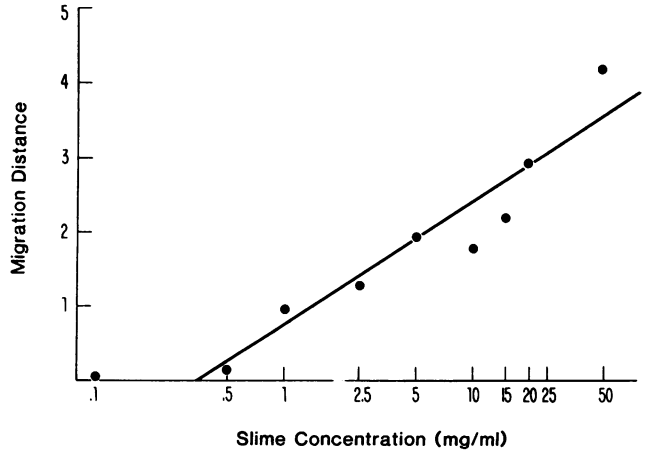

FIG. 1. PMN chemotaxis to *S. epidermidis* slime. The migration distance (chemotactic differential) is expressed in centimeters (magnification, ×40). PMN response to increasing concentrations of crude slime (0.1 to 50 mg/ml). Each point is the mean of at least three separate duplicate observations ($r = 0.943$).

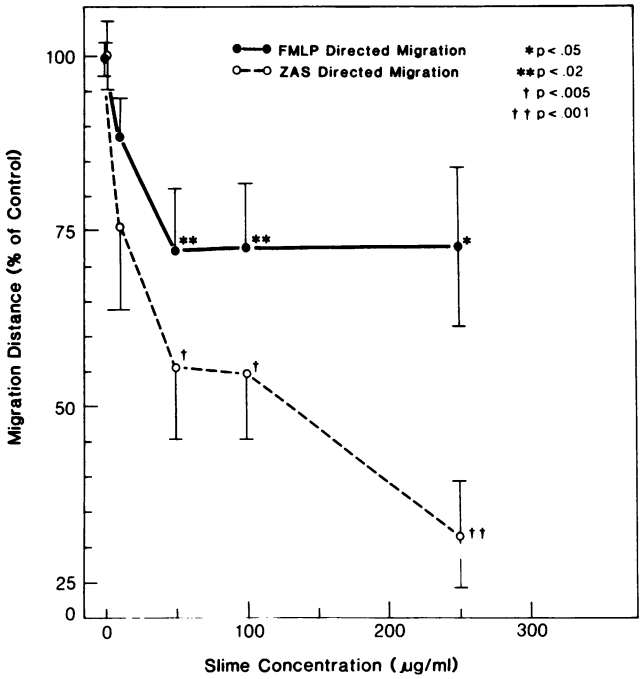

FIG. 2. Dose-response curves for inhibition of chemotactic response to ZAS and FMLP by *S. epidermidis* slime. PMNs were incubated in increasing concentrations of slime for 15 min at 37°C and washed. The migration distance (chemotactic differential) is expressed as the percentage of migration of the control PMNs incubated in GHBSS. ZAS and 10^{-8} M FMLP were used as chemoattractants. Each point is the mean ± the standard error of three separate experiments. P values are for migration of slime-incubated PMNs compared with the control-medium-incubated PMNs.

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 µg/ml, which was not significantly different, to 67.5 ± 6.8% at 250 µg/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-*

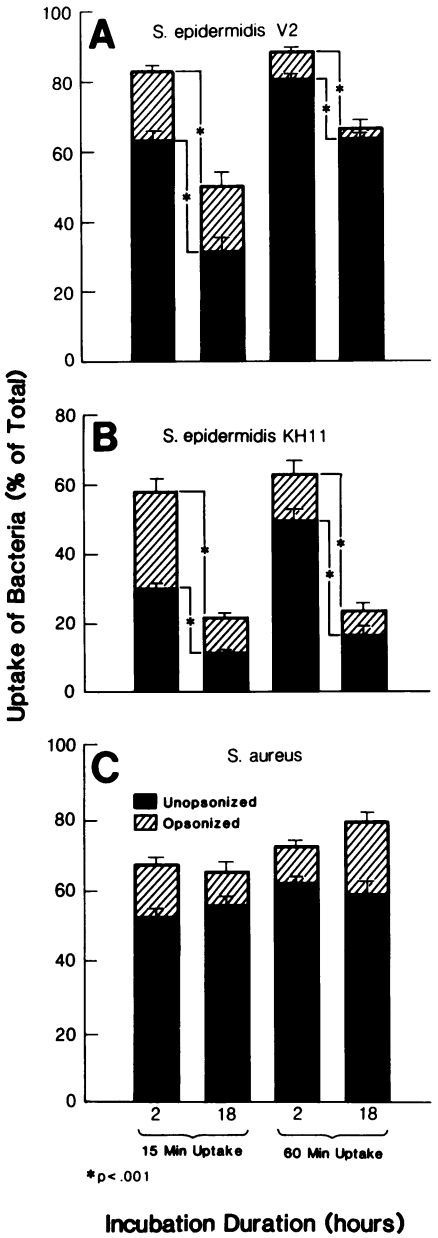

FIG. 3. PMN surface phagocytosis after 15 or 60 min of radiolabeled *S. epidermidis* V2 (A), KH11 (B), or *S. aureus* (C) adhered to plastic wells for 2 h or incubated in broth in plastic wells for 18 h. The uptake of bacteria by PMNs is presented as the percentage of the total adherent bacteria in the 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.

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.

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 andV2 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. PMN degranulation in response to expo-

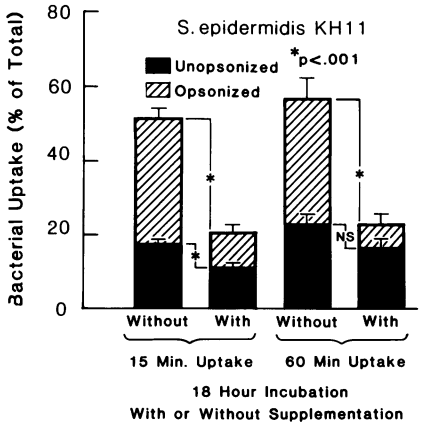

FIG. 4. Surface phagocytosis after 15 or 60 min of *S. epidermidis* KH11 incubated in plastic wells for 18 h in ^{3}H-labeled nutrient broth, with or without supplementation to promote slime production. The uptake of bacteria by PMNs is presented as the percentage of the total adherent bacteria in the 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.

sure 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.

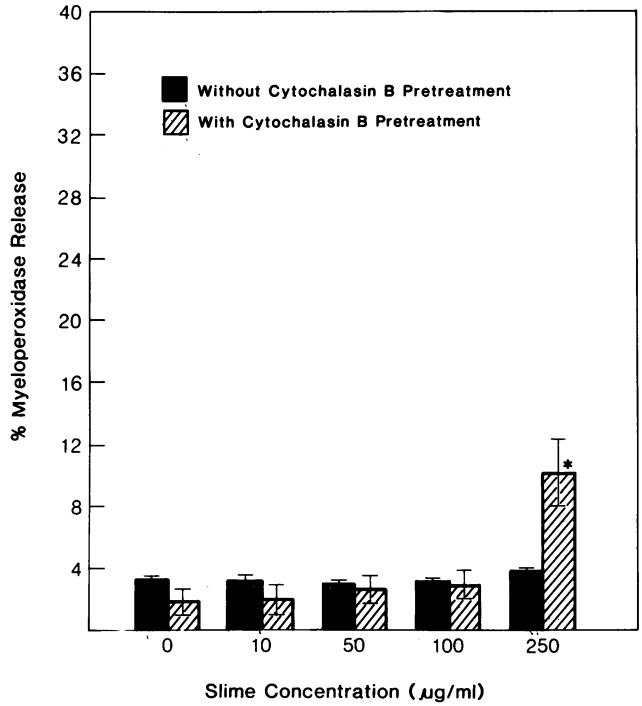

FIG. 5. PMN MPO release during a 30-min incubation with various concentrations of crude *S. epidermidis* slime with or without cytochalasin *b* (5 μg/ml) pretreatment. MPO release is expressed as a percentage of the total PMN MPO (*, $P < 0.01$ for PMNs incubated in crude slime compared with GHBSS-incubated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

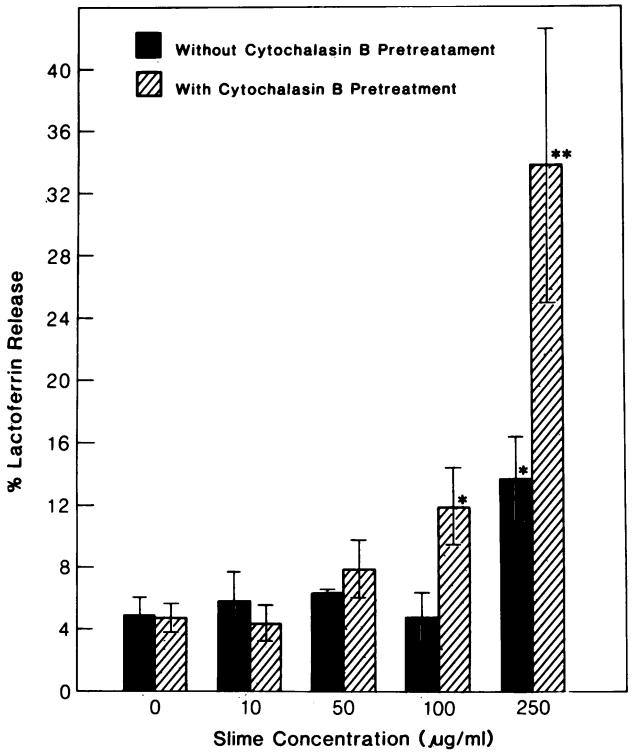

FIG. 6. PMN LF release during a 30-min incubation with various concentrations of crude *S. epidermidis* slime with or without cytochalisin *b* (5 μg/ml) pretreatment. LF release is expressed as a percentage of the total PMN LF (*, $P < 0.05$ and **, $P < 0.01$ for PMNs incubated in crude slime compared with GHBSS-incubated PMNs). Each point is the mean ± the standard error of at least three separate experiments.

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 chemoattractants, such as that associated with crude slime, were found to deactivate 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

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.

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LITERATURE CITED

1. **Archer, G. L.** 1978. Antimicrobial susceptibility and selection of resistance among *Staphylococcus epidermidis* isolates recovered from patients with infections of indwelling foreign devices. Antimicrob. Agents Chemother. **14**:353–359.
2. **Baumgart, S., S. E. Hall, J. M. Campos, and R. A. Polin.** 1983. Sepsis with coagulase-negative staphylococci in critically ill newborns. Am. J. Dis. Child. **137**:461–463.
3. **Bayston, R., and S. R. Penny.** 1972. Excessive production of mucoid substance in staphylococcus SII A, a possible factor in colonization of Holter shunts. Dev. Med. Child. Neurol. Suppl. **14**:25–28.
4. **Bender, J. W., and W. T. Hughes.** 1980. Fatal *Staphylococcus epidermidis* sepsis following bone marrow transplantation. Johns Hopkins Med. J. **146**:13–15.
5. **Bergman, M. J., R. L. Guerrant, F. Murad, S. H. Richardson, D. Weaver, and G. L. Mandell.** 1978. Interaction of polymorphonuclear neutrophils with *Escherichia coli*: effect of enterotoxin on phagocytosis, killing, chemotaxis and cyclic AMP. J. Clin. Invest. **61**:227–234.
6. **Bortolussi, R., P. Ferrieri, B. Björkstén, and P. G. Quie.** 1979. Capsular K1 polysaccharide of *Escherichia coli*: relationship to virulence in newborn rats and resistance to phagocytosis. Infect. Immun. **25**:293–298.
7. **Boxer, L. A., T. D. Coates, R. A. D. Haak, J. B. Wolach, S. Hoffstein, and R. L. Baehner.** 1982. Lactoferrin deficiency associated with altered granulocyte function. N. Engl. J. Med. **307**:404–410.
8. **Boyum, A.** 1968. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. Suppl. **21**:77–89.
9. **Callagen, R. P., S. J. Cohen, and J. T. Stewart.** 1961. Septicemia due to colonization of Spitz-Holter valves with staphylococci: five cases treated with methicillin. Br. J. Med. **1**:860–863.
10. **Chenoweth, D. E., J. R. Rowe, and T. E. Hugli.** 1979. A modified method for chemotaxis under agarose. J. Immunol. Methods **25**:337–352.
11. **Choo, M. H., D. R. Holmes, B. J. Gersh, J. D. Maloney, J. Merideth, J. R. Pluth, and J. Trusty.** 1981. Permanent pacemaker infections: characterization and management. Am. J. Cardiol. **48**:559–564.
12. **Christensen, G. D., A. L. Bisno, J. T. Parisi, B. McLaughlin, M. G. Hester, and R. W. Luther.** 1982. Nosocomial septicemia due to multiply antibiotic resistant *Staphylococcus epidermidis*. Ann. Intern. Med. **96**:1–10.
13. **Christensen, G. D., W. A. Simpson, A. L. Bisno, and E. H. Beachey.** 1982. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect. Immun. **37**:318–326.
14. **Christensen, G. D., W. A. Simpson, A. L. Bisno, and E. H. Beachey.** 1983. Experimental foreign body infections in mice challenged with slime-producing *Staphylococcus epidermidis*. Infect. Immun. **40**:407–410.
15. **Dismukes, E. W., A. W. Karchmer, M. J. Buckley, W. G. Austen, and M. N. Swartz.** 1973. Prosthetic valve endocarditis. Circulation **48**:365–377.
16. **Feld, R., W. D. Leers, J. E. Curtis, and D. E. Bergsagel.** 1975. Intravenous catheter infection study: a prospective trial in patients with neoplastic disease. Med. Pediatr. Oncol. **1**:175–181.
17. **Ferrante, A., and Y. H. Thong.** 1982. Separation of mononuclear and polymorphonuclear leukocytes from human blood by the one-step Hypaque-Ficoll method is dependent on blood column height. J. Immunol. Methods **48**:81–85.
18. **Fleer, A., R. C. Senders, M. R. Visser, R. P. Bijlmer, L. J. Gerards, C. A. Kraaijeveld, and J. Verhoef.** 1983. Septicemia due to coagulase-negative staphylococci in a neonatal intensive care unit: clinical and bacteriological features and contaminated fluids as a source of sepsis. Pediatr. Infect. Dis. **2**:426–431.
19. **Fletcher, M. P., B. E. Seligmann, and J. I. Gallin.** 1982. Correlation of human neutrophil secretion, chemoattractant receptor mobilization, and enhanced functional capacity. J. Immunol. **128**:941–948.
20. **Franson, T. R., N. K. Sheth, H. D. Rose, and P. G. Sohnle.** 1984. Scanning electron microscopy of bacteria adherent to intravascular catheters. J. Clin. Microbiol. **20**:500–505.
21. **Gemmel, C. G., M. Thelestam, and T. Wadström.** 1976. Toxigenicity of coagulase-negative staphylococci. Zentralbl.

Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **1976**(Suppl. 5):133–136.
22. **Gokal, R.** 1982. Peritonitis in continuous ambulatory peritoneal dialysis. J. Antimicrob. Chemother. **9**:417–422.
23. **Gray, E. D., G. Peters, M. Verstegen, and W. E. Regelmann.** 1984. Effect of extracellular slime substance from *Staphylococcus epidermidis* on the human cellular immune response. Lancet **i**:365–367.
24. **Gruer, L. D., R. Barlett, and G. A. J. Ayliffe.** 1984. Species identification and antibiotic sensitivity of coagulase-negative staphylococci from CAPD peritonitis. J. Antimicrob. Chemother. **13**:577–583.
25. **Henricks, P. A. J., M. E. van der Tol, R. M. W. M. Thyssen, B. S. van Asbeck, and J. Verhoef.** 1983. *Escherichia coli* lipopolysaccharides diminish and enhance cell function of human polymorphonuclear leukocytes. Infect. Immun. **41**:294–301.
26. **Henson, P. M., B. Sanolari, N. A. Schwartzman, and S. R. Hong.** 1978. Intracellular control of human neutrophil secretion. I. C5a-induced stimulus-specific desensitization and the effects of cytochalasin B. J. Immunol. **121**:851–855.
27. **Hetherington, S. V., J. K. Spitznagel, and P. G. Quie.** 1983. An enzyme-linked immunoassay (ELISA) for measurement of lactoferrin. J. Immunol. Methods **65**:183–190.
28. **Jupiter, J. B., A. W. Karchmer, J. D. Lowell, and W. H. Harris.** 1981. Total hip arthroplasty in treatment of adult hips with current or quiescent sepsis. J. Bone Jt. Surg. Am. Vol. **63**:194–200.
29. **Karchmer, A. W., G. L. Archer, and W. E. Dismukes.** 1983. *Staphylococcus epidermidis* causing prosthetic valve endocarditis: microbiologic and clinical observations as guides to therapy. Ann. Intern. Med. **98**:447–455.
30. **Lee, D. A., J. R. Hoidal, C. C. Clawson, P. G. Quie, and P. K. Peterson.** 1983. Phagocytosis by polymorphonuclear leukocytes of *Staphylococcus aureus* and *Pseudomonas aeruginosa* adherent to plastic, agar, or glass. J. Immunol. Methods **63**:103–114.
31. **Lemire, G. G., J. E. Morin, and A. R. C. Dobell.** 1975. Pacemaker infections: a 12-year review. Can. J. Surg. **18**:181–184.
32. **Locci, R., G. Peters, G. Pulverer.** 1981. Microbial colonization of prosthetic devices. III. Adhesion of staphylococci to lumina of intravenous catheters perfused with bacterial suspensions. Zentralbl. Bakteriol. Mikrobiol. Hyg. Abt. 1 Orig. B **173**:300–307.
33. **Lowder, J. N., H. M. Lazarus, and R. H. Herzig.** 1982. Bacteremias and fungemias in oncologic patients with central venous catheters. Arch. Intern. Med. **142**:1456–1459.
34. **Ludwicka, A., Y. Uhleubruch, G. Peters, P. N. Seug, E. D. Gray, J. Jeljaszewicz, and G. Pulverer.** 1984. Investigation on extracellular slime produced by *Staphylococcus epidermidis*. Zentralbl. Bakteriol. Mikrobiol. Hyg. Abt. 1. Orig. A **258**:256–267.
35. **Marrie, T. J., and J. W. Costerton.** 1984. Scanning and transmission electron microscopy of in situ bacterial colonization of intravenous and intraarterial catheters. J. Clin. Microbiol. **19**:687–693.
36. **Marrie, T. J., and J. W. Costerton.** 1984. Morphology of bacterial attachment to cardiac pacemaker leads and power packs. J. Clin. Microbiol. **19**:911–914.
37. **Masur, H., and W. D. Johnson.** 1980. Prosthetic valve endocarditis. J. Thorac. Cardiovasc. Surg. **80**:31–37.
38. **Munson, D. P., T. R. Thompson, D. E. Johnson, F. S. Rhame, N. VanDrunen, and P. Ferrieri.** 1982. Coagulase-negative staphylococcal septicemia: experience in a newborn intensive care unit. J. Pediatr. **101**:602–605.
39. **Musher, D. M., H. A. Verbrugh, and J. Verhoef.** 1981. Suppression of phagocytosis and chemotaxis by cell wall components of *Staphylococcus aureus*. J. Immunol. **127**:84–88.
40. **Nelson, R. D., R. T. McCormack, V. D. Fiegel, and R. L. Simmons.** 1978. Chemotactic deactivation of human neutrophils: evidence for nonspecific and specific components. Infect. Immun. **22**:441–444.
41. **Nelson, R. D., P. G. Quie, and R. L. Simmons.** 1975. Chemotaxis under agarose: a new and simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocytes and monocytes. J. Immunol. **115**:1650–1656.
42. **Patterson, F. P., and C. S. Brown.** 1972. The McKee-Farrar total hip replacement: preliminary results and complications of 368 operations performed in five general hospitals. J. Bone Jt. Surg. Am. Vol. **54**:257–275.
43. **Peters, G., R. Locci, and G. Pulverer.** 1981. Microbial colonization of prosthetic devices. II. Scanning electron microscopy of naturally infected intravenous catheters. Zentralbl. Bakteriol. Mikrobiol. Hyg. Abt. 1 Orig. B **173**:293–299.
44. **Peters, G., R. Locci, and G. Pulverer.** 1982. Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. J. Infect. Dis. **146**:479–482.
45. **Peters, G., F. Saborowski, R. Locci, and G. Pulverer.** 1984. Investigations on staphylococcal infection of transvenous endocardial pacemaker electrodes. Am. Heart J. **108**:359–365.
46. **Peterson, P. K., B. J. Wilkinson, Y. Kim, D. Schmeling, and P. G. Quie.** 1978. Influence of encapsulation on staphylococcal opsonization and phagocytosis by human polymorphonuclear leukocytes. Infect. Immun. **19**:943–949.
47. **Rubin, J., W. A. Rogers, H. M. Taylor, E. D. Everett, B. F. Prownat; L. V. Frato, and K. D. Nolph.** 1980. Peritonitis during continuous ambulatory peritoneal dialysis. Ann. Intern. Med. **92**:7–13.
48. **Sattler, F. R., J. B. Foderaro, and R. C. Aber.** 1984. *Staphylococcus epidermidis* bacteremia associated with vascular catheters: an important cause of febrile morbidity in hospitalized patients. Infect. Control (Thorofare) **5**:279–283.
49. **Schimke, R. T., P. H. Black, V. H. Mark, and M. N. Swartz.** 1961. Indolent *Staphylococcus albus* or *aureus* bacteremia after ventriculotriostomy. N. Engl. J. Med. **264**:264–270.
50. **Schmeling, D. J., G. C. Gemmell, P. R. Craddock, P. G. Quie, and P. K. Peterson.** 1981. Effect of staphylococcal alpha-toxin on neutrophil migration and adhesiveness. Inflammation **5**:313–322.
51. **Schoenbaum, S. C., P. Gardner, and J. Shillito.** 1975. Infection of cerebrospinal fluid shunts: epidemiology, clinical manifestations and therapy. J. Infect. Dis. **131**:543–552.
52. **Seligmann, B. E., M. P. Fletcher, and J. I. Gallin.** 1982. Adaptation of human neutrophil responsiveness to the chemoattractant *N*-formylmethionylleucylphenylalanine: heterogeneity and/or negative cooperative interaction of receptors. J. Biol. Chem. **257**:6280–6286.
53. **Shurleff, D. B., E. L. Foltz, R. D. Weeks, and J. Loeser.** 1974. Therapy of *Staphylococcus epidermidis*: infections associated with cerebrospinal fluid shunts. Pediatrics **53**:55–62.
54. **Smith, C. W., J. C. Hollers, R. A. Patrick, and C. Hassett.** 1979. Motility and adhesiveness in human neutrophils: effects of chemotactic factors. J. Clin. Invest. **63**:221–229.
55. **Speller, D. C. E., and R. G. Mitchell.** 1973. Coagulase-negative staphylococci causing endocarditis after cardiac surgery. J. Clin. Pathol. **16**:517–522.
56. **Turner, W. H., and D. J. Pickard.** 1979. Immunological relationship between delta-hemolysins of *Staphylococcus aureus* and coagulase-negative strains of staphylococci. Infect. Immun. **23**:910–911.
57. **VanDyke, T. E., E. Bartholomew, R. J. Genco, J. Slots, and M. J. Levine.** 1982. Inhibition of neutrophil chemotaxis by soluble bacterial products. J. Peridontol. **53**:502–508.
58. **Van Epps, D. E., and M. L. Garcia.** 1980. Enhancement of neutrophil function as a result of prior exposure to chemotactic factor. J. Clin. Invest. **66**:167–175.
59. **Vas, S. I.** 1983. Microbiologic aspects of chronic ambulatory peritoneal dialysis. Kidney Int. **23**:83–92.
60. **Verhoef, J., D. M. Musher, J. S. Spika, H. A. Verbrugh, and F. C. A. Jaspers.** 1983. The effect of staphylococcal peptidoglycan on polymorphonuclear leukocytes in vitro and in vivo. Scand. J. Infect. Dis. Suppl. **41**:79–85.
61. **Wacker, W. E. C., D. D. Ulmer, and B. L. Vallee.** 1956. Malic and lactic dehydrogenase activities and zinc concentrations in serum. N. Engl. J. Med. **255**:449–456.

62. **Wade, J. C., S. C. Schimpff, K. A. Newman, and P. H. Wiernick.** 1982. *Staphylococcus epidermidis*: an increasing cause of infection in patients with granulocytopenia. Ann. Intern. Med. **97**:503–508.
63. **Wexler, D. E., R. D. Nelson, and P. P. Cleary.** 1983. Human neutrophil chemotactic response to group A streptococci: bacteria-mediated interference with complement-derived chemotactic factors. Infect. Immun. **39**:239–246.
64. **Wilson, P. D., E. A. Salvati, P. Aglietti, and L. J. Kutner.** 1973. The problem of infection in endoprosthetic surgery of the hip joint. Clin. Orthop. Relat. Res. **96**:213–221.
65. **Winston, D. J., D. V. Dudnick, M. Chapin, W. G. Ho, R. P. Gale, and W. J. Martin.** 1983. Coagulase-negative staphylococcal bacteremia in patients receiving immunosuppressive therapy. Arch. Intern. Med. **143**:32–36.
66. **Zimmerli, W., P. D. Lew, and F. A. Waldvogel.** 1984. Pathogenesis of foreign body infection: evidence for a local granulocyte defect. J. Clin. Invest. **73**:1191–1200.