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Persistent In Vitro Survival of Coagulase-Negative Staphylococci Adherent to Intravascular Catheters in the Absence of Conventional Nutrients

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The in vitro survival of coagulase-negative staphylococci in media devoid of routine nutritional supplementation was assessed in the presence and absence of catheter materials to evaluate bacterium-device interactions. Strains of slime- and non-slime-producing coagulase-negative staphylococci were suspended in phosphate-buffered saline together with multiple segments of polyvinyl chloride (PVC), Teflon, Silastic, and polyurethane catheters and in control suspensions without catheters. Catheters were removed at 2 min and 24, 48, 72, and 96 h of incubation and washed thoroughly, and semiquantitative roll cultures were performed on blood agar. In addition, after 96 h catheters were introduced into tryptic soy broth (TSB), and roll cultures were performed after 18 h of incubation. Results demonstrated that after 96 h, 6 of 32 catheter specimens (4 PVC) had >10 CFU of coagulase-negative staphylococci per catheter; after TSB addition, 18 of 32 catheter specimens had ≥100 CFU per catheter (8 of 8 PVC catheters had >1,000 CFU per catheter). In control suspensions, no growth was seen at 96 h or after TSB addition. No differences in the survival of slime- versus non-slime-producing strains were observed in control or catheter studies. These findings suggest that both slime- and non-slime-producing coagulase-negative staphylococci survive in vitro on catheters (especially PVC) in the absence of conventional nutrients and can proliferate on catheters when nutrients are added. Catheter-adherent coagulase-negative staphylococci appear to possess survival mechanisms under adverse conditions which may relate to the genesis of occult foreign-body-associated infections.

Intravascular catheter-associated infections are a significant source of nosocomial morbidity and mortality. The exact mechanisms by which these infections develop are not fully defined, but the phenomenon of bacterial adherence to foreign body materials appears to be a critical factor in initiating infections in a scheme in which adhesion precedes and promotes device colonization, with subsequent infection (4, 11, 13, 16, 18).

Coagulase-negative staphylococci appear to be infrequent pathogens when not associated with foreign devices. However, in the presence of devices such as prosthetic vascular grafts (1), prosthetic heart valves (9), vascular catheters (2, 14), and orthopedic (5, 7) or neurosurgical (8, 15) implants, coagulase-negative staphylococci have been increasingly appreciated as important pathogens. In these settings, the organisms may gain access to the device at the time of surgery or catheter placement, but infection may not become clinically evident for days to months after the initial contamination event (1, 7, 10). Conversely, with vascular catheters which exit externally through the skin, coagulase-negative staphylococci may colonize the skin-device interface, migrate along the catheter tract subcutaneously, and eventually cause infection of the intravascular device (11). Certain conditions may then perpetuate the survival of coagulase-negative staphylococci on such devices. These organisms, when attached to the surfaces of foreign bodies, may produce an extracellular slime which solidifies the attachment, prevents the access of effective antimicrobial agents to the cell surface, thereby allowing for the persistence of coagulase-negative staphylococci on catheters (17), and aids in resistance against cellular host defenses (6). It is unclear whether innate bacterial factors alone are responsible for adherence and survival or whether additional bacterium-device interactions or other factors also participate in these processes.

This study was designed to assess the survival of coagulase-negative staphylococci in media devoid of routine in vitro nutritional supplementation in the presence and absence of various catheter materials to further delineate bacterium-device interactions.

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MATERIALS AND METHODS

Organisms. Four strains of coagulase-negative staphylococci were used for this study. Two clinical isolates each of slime- and non-slime-producing coagulase-negative staphylococci were chosen and characterized for slime production by visual observation of slime production after growth in tryptic soy broth (TSB) (Difco Laboratories, Detroit, Mich.) in glass tubes and confirmation by alcian blue staining of the tubes (3, 4). One strain each of slime- and non-slime-producing coagulase-negative staphylococci were furnished by G. D. Christensen, University of Tennessee College of Medicine, Memphis. Each strain was grown for 2 to 4 h at 36°C in TSB, washed three times in phosphate-buffered saline (PBS, pH 7.2), and suspended at 10^6 CFU/ml in PBS by use of a 0.5 McFarland $BaSO_4$ turbidity standard. Inocula were confirmed by aliquot sampling and dilutions.

Catheters. Four types of sterile commercial intravascular devices were used: polyvinyl chloride (PVC) (Swan-Ganz; American Edwards Laboratories, Santa Ana, Calif.), Teflon (Quik-Cath; Travenol Laboratories, Deerfield, Ill.), Silastic

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TABLE 1. Organism survival on catheters

Catheter type[a]	No. of catheter roll cultures positive (>10 CFU/catheter) in phase[b]:				
	1 at:				2 [range of CFU/catheter]
	24 h	48 h	72 h	96 h	
PVC	6 (4)	5 (3)	5 (3)	4 (2)	8 (4) [>1,000]
Teflon	6 (3)	2 (1)	1 (1)	0	4 (2) [100–>1,000]
Silastic	4 (2)	4 (2)	4 (2)	2 (1)	4 (2) [>1,000]
Polyurethane	6 (3)	4 (2)	2 (1)	0	2 (1) [700–>1,000]

[a] Eight catheters of each type were tested.
[b] Data represent the cumulative number of slime- and non-slime-producing strains tested which were adherent to catheters. Data in parentheses represent the number of slime-producing strains tested which were adherent to catheters.

(Broviac; Evermed, Medina, Wash.), and polyurethane (Intracath; Deseret Pharmaceuticals, Sandy, Utah). All devices were cut into 1-cm segments and gas sterilized.

Media. PBS, FA buffer (consisting of 0.85% saline buffered to pH 7.2; Difco Laboratories), and TSB were prepared in sterile fashion by following the standard instructions of the manufacturers.

Study phase 1. Parallel sets of sterile tubes were used for each catheter material type and each study strain. The experimental tubes contained 5 ml of washed, log-phase coagulase-negative staphylococci suspended at 10^6 CFU/ml in PBS, into which were introduced multiple 1-cm catheter segments. The control tubes contained only the organisms in PBS. All tubes were incubated at 36°C. For the experimental group, catheter segments were removed at 2 min and at 24, 48, 72, and 96 h of incubation, washed in running water for 2 min, shaken to remove excess fluid, and rolled across the surfaces of blood agar plates as described by Maki et al. (12). For the control group, 0.01-ml aliquots were removed from suspensions at the same time and streaked across the surfaces of blood agar plates. The control inocula were validated as comparable to the experimental inocula by a previously described dye technique (18) to quantitate the amount of fluid carried on the catheter surface. All blood agar plates were then incubated for 18 h at 36°C, and resultant colonies were visually counted by using a Quebec Colony Counter (Spencer Lens Co., Buffalo, N.Y.). All studies were performed in duplicate.

Study phase 2. For the experimental group, additional catheter segments were removed at 96 h, washed, shaken to remove excess fluid, and individually introduced into 10 ml of TSB. After incubation for 18 h at 36°C, catheters were washed and rolled across the surfaces of blood agar plates as described above. For the control group, 0.01-ml aliquots were inoculated into 10 ml of TSB at 96 h, incubated for 18 h at 36°C, visually observed for turbidity, and further processed by plating 0.001-ml aliquots on blood agar plates for quantitative counts.

All blood agar plates with no growth were observed for an additional 48 h at 36°C to look for slower-growing organisms. Coagulase-negative staphylococci recovered at 96 h on blood agar were identified by the Staph-Ident (Analytab Products, Plainview, N.Y.) and tested for drug susceptibility by the routine Kirby-Bauer procedure performed to establish similarities or differences from the original strains.

RESULTS

A summary of the results of study phases 1 and 2 is shown in Table 1. At 72 h of incubation, 12 of 32 catheter specimens had >10 CFU of coagulase-negative staphylococci per catheter; at 96 h only 6 of 32 were positive by this criterion (4 of which were PVC catheters). After TSB addition, 18 of 32 catheter specimens had ≥100 CFU per catheter (8 of 8 PVC catheters had >1,000 CFU per catheter).

For control suspensions, 10 of 32 tubes had ≥10 CFU of coagulase-negative staphylococci per ml at 48 h and no growth at 96 h on plates or after TSB addition. No remarkable differences in the survival of slime- versus non-slime-producing coagulase-negative staphylococci in control suspensions could be observed at any of the times tested.

Various morphotypes of coagulase-negative staphylococci were observed on blood agar when roll cultures of catheters with adherent bacteria were performed; small numbers were also seen in the control suspensions. Small-colony morphotypes usually appeared after prolonged incubation.

DISCUSSION

The results of this study demonstrate that both slime- and non-slime-producing strains of coagulase-negative staphylococci can survive on catheters in the absence of conventional nutrients and can proliferate on catheters when nutrients are added. In particular, coagulase-negative staphylococci adhering to PVC catheters appear to be particularly capable of survival under adverse conditions. Previous studies have demonstrated that coagulase-negative staphylococci have a higher affinity for PVC than for other catheter types clinically (16) and in vitro (18), and it is possible that adherence phenomena are highly variable, depending on catheter substrates. The absence of uniform growth on PVC at 72 and 96 h on roll culture plates prior to nutrient addition may reflect bacteria entering a state of "dormancy" that cannot be detected by routine roll culture techniques. The finding on blood agar of smaller, slower-growing colonies giving a variety of morphotypes appears to relate to "dormant," metabolically slower organisms. No differences in the survival of individual strains with regard to the presence or absence of slime production in the four strains tested were apparent, although slime-producing strains showed quantitatively greater survival on catheters than did non-slime-producing strains.

These findings suggest that adherent coagulase-negative staphylococci may have survival advantages under adverse conditions which are not entirely dependent on slime production. Peters and colleagues (13) suggested that coagulase-negative staphylococci may be able to use catheter materials as a nutrient source. It may also be possible that adherence to the surfaces of foreign objects may allow organisms to become metabolically dormant; thus, they may persist on foreign body materials in an occult fashion until a clinical infection develops. Regardless of the mechanisms involved, these observations imply that factors other than slime production contribute to the pathogenicity of coagulase-negative staphylococci in association with prosthetic devices. However, after initial adherence, the presence of various amounts of slime enveloping catheter-adherent bacteria is likely to form an impervious barrier against in vivo nutrients, further allowing organisms to persist in growth-limiting environment, a status comparable to our in vitro experimental system.

The interaction of coagulase-negative staphylococci and foreign body materials appears to be complex, and many factors likely contribute to the ability of the organisms to attach to medical devices, persistently survive, evade host defenses or treatment, and cause serious infections. The

survival features described here may relate to the clinical observation that coagulase-negative staphylococcus–foreign body infections such as those involving prosthetic orthopedic joints (7) and vascular implants (1) sometimes become evident only after long periods (i.e., months to years) from the time of initial contamination. These observations will require further characterization by more extensive in vitro and clinical investigations to define the exact mechanisms of pathogenesis involved in this complex process.

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