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NOTES

Experimental Foreign Body Infections in Mice Challenged with Slime-Producing *Staphylococcus epidermidis*

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The virulence of two previously described *Staphylococcus epidermidis* strains was examined in an experimental model of foreign body infection in mice. Animals challenged with the slime-producing strain developed three times as many infections as animals challenged with the strain that did not produce slime ($P < 0.001$). Bacterial isolates recovered from the infected sites retained the characteristics of the inoculated strain. Animals without foreign bodies but challenged in a similar manner with either staphylococcal strain did not become infected. Thus, the presence of a foreign body predisposed the animals to *S. epidermidis* infection. These results indicate that the production of slime by *S. epidermidis* is a stable characteristic retained after animal passage and may be important in the pathogenesis of these infections.

Staphylococcus epidermidis (Baird-Parker) is the paradigm of the opportunistic pathogen. Normally a human saprophyte, it is notable for producing infections when the host is compromised by the presence of a deep-tissue foreign body such as a prosthetic cardiac valve (1), orthopedic applicance (12), cerebrospinal fluid shunt (9), or intravascular catheter (4).

While investigating an outbreak of intravascular catheter-associated sepsis due to *S. epidermidis* (4), we noted that some strains of these organisms when grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) formed a viscid film adherent to the walls of the test tube (5). The slimy growth appeared to be related to the attachment of these organisms to smooth surfaces in general (5). In addition, we noted that slime-producing strains were epidemiologically associated with intravascular catheter sepsis (5). These studies suggested that the ability to attach to a foreign body and colonize it was important in the pathogenesis of foreign body infections by *S. epidermidis*. As the first step in testing this hypothesis, we have developed a murine model of foreign body infection and have examined the virulence of a slime-producing (RP-12) and a slime nonproducing (SP-2) strain of *S. epidermidis* (5).

Both strains were gram-positive clustering cocci that were catalase positive, coagulase negative, and sensitive to novobiocin. Both strains fermented glucose but not mannitol. By the criteria of Baird-Parker (3) they were considered *S. epidermidis*. The slime-producing strain, RP-12, was first isolated from the blood of a patient with intravascular catheter-associated *S. epidermidis* sepsis, and the slime-nonproducing strain, SP-2, was isolated from a blood culture of a patient without symptoms of an *S. epidermidis* infection and was a presumed blood culture contaminant (4). RP-12 was susceptible to cephalothin, chloramphenicol, and tetracycline and resistant to penicillin, oxacillin, clindamycin, erythromycin, and kanamycin. SP-2 was susceptible to oxacillin, cephalothin, clindamycin, erythromycin, gentamicin, and tetracycline and resistant to penicillin, chloramphenicol, and kanamycin. Stock cultures of both strains were first stored in outdated human blood at −70°C and later as lyophilized cultures. Working cultures were maintained on Trypticase soy agar with 5% sheep blood. The bacterial inocula were prepared by propagating the organisms overnight at 37°C in Trypticase soy broth without glucose supplementation; this culture medium was used to avoid clumping of slime-producing organisms. Trypticase soy broth without glucose was assembled from its components, phytone and tryptone (BBL Microbiology Systems). The bacteria were washed once and suspended with

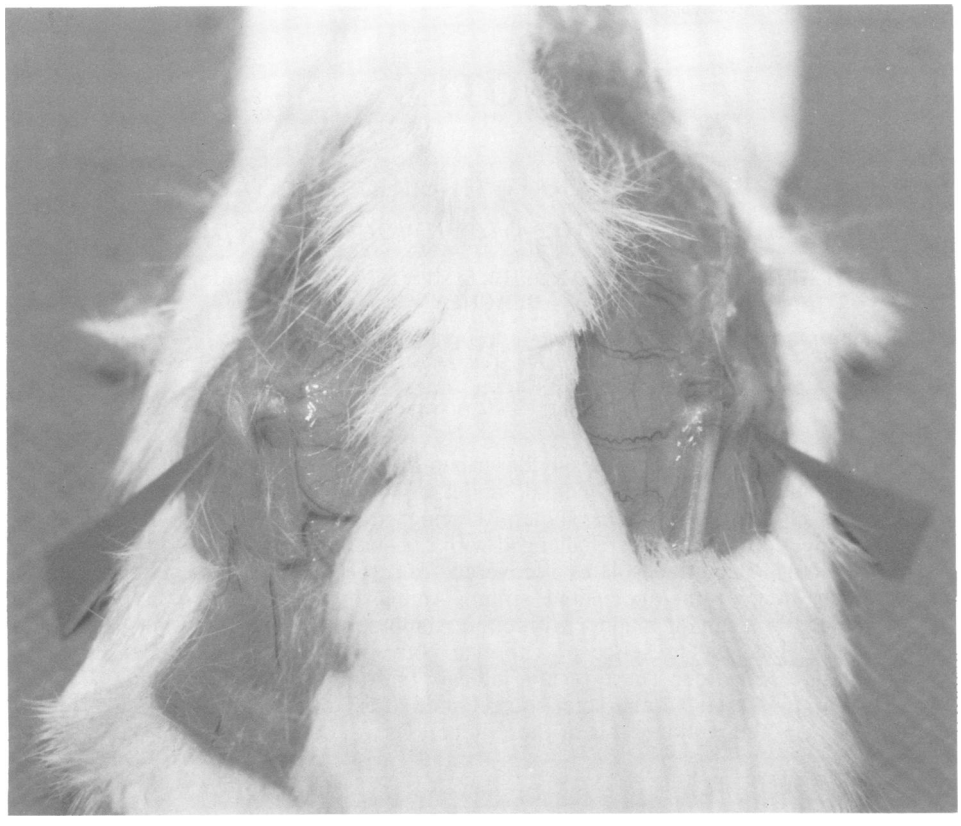

FIG. 1. Mouse with two infected catheters. The catheters have abscesses at their ends and surrounding erythema and adhesions. Two brands of catheters were used in equal numbers, and the rates of infection were the same for each brand.

phosphate-buffered saline in 1/10 of the original volume, resulting in 10^{10} CFU per ml. A 0.1-ml amount was injected as the inoculum.

Swiss albino mice (25 to 30 g) were prepared by cleansing the fur with povidone-iodine solution (Pharmadine; Sherwood Pharmaceutical Co., Mahwah, N.J.) followed by the aseptic insertion of 1-cm lengths of a 14-gauge plastic intravascular catheter into the subcutaneous area of each flank; the wound was closed with a single silk suture. Control mice underwent a similar procedure, but the catheter was withdrawn from the animal before closure of the wound. Two catheter brands, Intracath (Deseret, Sandy, Utah) and Radiopaque Catheter (C. R. Bard, Murray Hill, N.J.), from various lots were used. After a 3-day rest, the animals were culled for mice with unhealed wounds. The healthy mice had the inoculum injected subcutaneously at the site of the foreign body. Control animals were simply injected subcutaneously over the flanks at the site of the sham procedure. In one trial, animals were prepared with one flank having a foreign body and the other a sham operation. Ten days later, the animals were sacrificed, and the catheters were cultured. The skin of the control animals was stripped from their flanks, and the flanks were vigorously swabbed with a sterile cotton applicator. In no case was an abscess apparent.

In addition, two groups of 12 animals each were injected intraperitoneally with 10^9 RP-12 or SP-2 and sacrificed 4 weeks later. The peritoneal cavity was also swabbed with a cotton applicator.

All swabs and catheters were cultured in Trypticase soy broth for 1 to 3 days. Tubes showing growth were streaked out onto Trypticase soy agar with 5% sheep blood. White or grey colonies that were gram-positive cocci and catalase positive were tested for antimicrobial susceptibility by the disk diffusion method. Isolates with antimicrobial susceptibilities similar to those of the parent strain were counted as representing an infection of the animal and the catheter. These isolates were tested for slime production by lightly inoculating a tube of Trypticase soy broth and, after overnight incubation at 37°C, examining the tube for the presence or absence of a film lining the walls of the test tube.

TABLE 1. Incidence of *S. epidermidis* infection

Expt group	No. infected/no. challenged	
	RP-12	SP-2
Animals[a]		
Experimental	34/64	11/64
Control	0/52	0/53
Catheters[b]		
Experimental	44/99	12/94
Control	0/78	0/81

[a] For RP-12 versus SP-2 animal infections, $P < 0.001$ by chi-square analysis.
[b] For RP-12 versus SP-2 catheter infections, $P < 0.001$ by chi-square analysis.

TABLE 2. Stability of strain markers through animal passage

Marker[a]	Antimicrobial susceptibility, zone of inhibition (mm ± SD)	
	RP-12 (N = 44)	SP-2 (N = 12)
Penicillin	6	20.6 ± 1.4
Oxacillin	6	20.7 ± 1.6
Cephalothin	26.2 ± 2.1	34.8 ± 1.2
Clindamycin	6	28.5 ± 2.8
Erythromycin	6	28.5 ± 3.2
Gentamicin	6	15.7 ± 1.4
Kanamycin	6	6.9 ± 2.2
Chloramphenicol	18.3 ± 2.7	6
Tetracycline	18.9 ± 1.6	21.0 ± 2.0

[a] For RP-12, 44 isolates were positive for slime production and zero were negative. For SP-2, no isolates were positive for slime production and 12 strains were negative.

Both strains of *S. epidermidis*, when injected around the site of the subcutaneous catheter, produced abscesses (Fig. 1). It was apparent that the foreign body impaired the ability of the animal to clear these strains, since neither strain produced an infection when injected subcutaneously into the sham-operated control mice (Table 1) or when injected intraperitoneally.

To eliminate individual animal variations and to control for possible systemic effects caused by the catheter implantation, 65 animals were implanted with catheters on one flank and sham operated on the opposite flank. Again, none of the sham-operated sites became infected. The rate of catheter infections in this group was the same as that for animals implanted with two subcutaneous catheters, and the data have been combined (Table 1). A total of 53% of the mice injected with the slime-producing strain (RP-12) were found to have catheter infections, whereas only 17% of the mice challenged with slime-nonproducing strain (SP-2) developed infections (Table 1) ($P < 0.001$).

The identity of the RP-12 and SP-2 strains recovered from infected animals was established by their antimicrobial susceptibility patterns. These markers remained stable through the course of the infection (Table 2). We did not detect spontaneous curing of antibiotic resistance. The capacity or incapacity to produce slime was also stable through animal passage. The 44 recovered RP-12 isolates retained their capacity to produce slime, whereas the 12 recovered SP-2 isolates lacked this ability (Table 2).

Previous studies of animal models of *S. epidermidis* infections have demonstrated the requirement of large bacterial inocula and either an unusual bacterial strain or a compromised host. Namavar et al. (8) used 10^6 to 10^8 CFU infected intracerebrally into neonatal mice to establish the 50% lethal doses for their strains. Lowy et al. (7) used 3.5×10^9 CFU injected intraperitoneally with hog gastric mucin into newly weaned (15-g) mice to produce peritonitis. In addition, the strain used by Lowy et al., Sut, appeared to be unusually virulent, as described by Vazquez and Archer (11). Other unusual strains with increased pathogenic capacity have been reported by Thorig et al. (a Baird-Parker biotype S6 strain) (10), Ichiman and Yoshida (6), and Yoshida and Minegishi (13). In two separate studies, Ichiman and Yoshida and Yoshida and Minegishi screened 380 clinical isolates (6, 13) to find 14 strains that could produce mouse peritonitis without the aid of hog gastric mucin when 10^9 CFU were injected intraperitoneally. As opposed to most *S. epidermidis* strains, these unusually virulent strains were found to be encapsulated. Encapsulation does not appear, however, to be the same as slime production, since neither RP-12 nor SP-2 was mouse virulent when injected intraperitoneally, and in our hands, ATCC 31432 (one of the encapsulated strains of Ichiman and Yoshida) did not produce slime. Most animal model investigations with *S. epidermidis* have used rabbits compromised by the catheter induction of nonbacterial thrombotic endocarditis (2, 10, 11). The infecting dose has been high, varying between 10^7 CFU for the Thorig et al. Baird-Parker S6 strain (10) to 5×10^9 for the Lowy Sut strain (2, 11) and other *S. epidermidis* strains (11).

Our model confirmed the clinically noted association of foreign bodies and *S. epidermidis* infections. Both *S. epidermidis* strains produced infections only in the presence of a foreign body. Apparently, the foreign body interferes with the host's clearance of these normally avirulent organisms. In a recent similar study in rabbits, Zimmerli et al. (14) noted that the presence of a subcutaneous plastic tissue cage was required to establish an infection with the relatively avirulent *Staphylococcus aureus* strain Wood 46.

Further investigations on their part established that in the presence of the foreign body, phagocytosis was impaired.

The two strains examined in this model were chosen because of their previously described capacity or incapacity to produce slime. In this regard, the differences in their virulence are noteworthy, since they suggest that, in addition to host factors, i.e., the foreign body, bacterial factors are important in pathogenesis. Although RP-12 is a demonstrated pathogen and SP-2 is a suspected cultural contaminant, the greater virulence of the RP-12 organism is consistent with the hypothesis that slime production is important in the pathogenesis of foreign body infections by encouraging colonization or discouraging phagocytosis.

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