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Bacterial Concentration Correlations in Experimental Endocarditis Caused by *Staphylococcus epidermidis*

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Using 13 strains of *Staphylococcus epidermidis* to produce catheter-induced experimental endocarditis in rats, we found that bacterial concentrations in blood cultures obtained at the time of sacrifice correlated significantly with the number of organisms per gram of endocardial vegetation ($P < 0.001$) and the total number of organisms per vegetation ($P < 0.001$). Furthermore, blood culture concentrations correlated with vegetation weights ($P < 0.001$) and sizes of infecting inocula ($P < 0.0001$). Mean bacterial concentrations in vegetations more than doubled as bacterial concentrations in blood rose from less than 10 to greater than 100 CFU/ml. Mean values for vegetation weights, total organisms per vegetation, and sizes of infecting inocula were also reflected by the intensity of bacteremia. Moreover, intracardiac catheters were more likely colonized as bacterial concentrations in blood cultures increased, with all catheters culture positive in the 25 animals that exhibited high-grade bacteremia (≥100 CFU/ml). Slime production by the bacteria did not influence the above-mentioned correlations. These data indicate that the blood concentration of bacteria reflects the microbiologic status of infected vegetations in experimental infective endocarditis.

In a landmark study (4), Beeson and colleagues demonstrated several important features of bacteremia associated with infective endocarditis in humans by sampling blood from multiple sites, including the femoral artery, inferior vena cava, hepatic vein, right atrium, and extremity veins. First, infected vegetations released an almost constant number of organisms, and second, the level of bacteremia produced by endocardial lesions remained "remarkably constant." It was therefore concluded that practically all blood cultures obtained from untreated endocarditis patients should be culture positive.

Although the experimental model of endocarditis has been used extensively to examine such aspects of infective endocarditis as pathogenesis (2, 17), prophylaxis (1, 12), and therapy (14, 18), data concerning the relationship between level of bacteremia and concentration of organisms in vegetation tissue are extremely limited (7, 17). We therefore report findings from 299 animal experiments with 13 different strains of *Staphylococcus epidermidis* in the production of experimental endocarditis in the rat model. We observed striking correlations between bacterial concentrations in blood cultures obtained at the time of sacrifice and several microbiologic characteristics of infected vegetations and indwelling catheters.

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MATERIALS AND METHODS

Microorganisms. All 13 strains were isolates from humans and were designated as *S. epidermidis*, per manufacturer instructions, with two commercially available systems: the API Staph-Ident System and the API Staph-Trac System (Analytab Products, Plainview, N.Y.). Seven strains have been fully detailed in prior reports (2, 5). Two other strains, RP62A-NA and RP62A-NAR, were isolated from the parent strain, RP62A, by serial centrifugation techniques described elsewhere (G. D. Christensen, L. M. Baddour, and W. A. Simpson, submitted for publication). Except for changes in the amount of surface slime present, as seen on electron microscopy, and in vitro adherence characteristics, these two strains were very similar to the parent strain of *S. epidermidis*. The four remaining strains were obtained from skin (K5 and K15) and blood (RP39 and SP27).

Production of endocarditis and bacteriologic techniques. Details of the production of experimental endocarditis in the rat model have been described elsewhere (2). Briefly, 48 h after left-heart catheterization via the right common carotid artery, male Wistar rats were intravenously injected with various inocula of *S. epidermidis*. Four days later, the animals were sacrificed, and blood cultures were obtained just before sacrifice. Blood (3 to 5 ml) was generally obtained by direct external jugular vein puncture. One milliliter was taken for quantitation, being spread onto the surface of a 150-mm (diameter) plate containing 70 ml of Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.).

Vegetations, once resected, were weighed (wet) and homogenized with a mortar and pestle in 1 ml of phosphate-buffered saline (PBS). Dilutions of the homogenate were then made in PBS for quantitative colony counts and plated onto Trypticase soy agar that had been supplemented with 5% sheep blood (BBL). The portion of vegetation suspension that was not used in the dilution process was cultured in Trypticase soy broth.

Indwelling catheters were also resected and placed directly into tubes filled with Trypticase soy broth for culture. No quantitation of catheter colonization was attempted.

The antibiotic susceptibilities of *S. epidermidis* strains isolated from blood, catheter, and vegetation cultures were confirmed as being identical to that of the challenge strain by the Kirby-Bauer diffusion method (3). Slime production was checked as previously described (6). Statistical analysis was performed with the SAS Statistical Package (Cary, N.C.) based on the equation of Kendall Tau B for coefficient correlations.

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TABLE 1. Characteristics of the 13 strains of *S. epidermidis* used in this study

Strain	Clinical source	No. of infected rats	Slime production[a]	Inoculum range (CFU)
RP62A	Catheter sepsis	85	+	10^5–10^9
RE19	Nasal isolate from nurse	52	+	10^4–10^7
RP62A-NAR	Daughter strain of RP62A	17	+	10^5–10^7
RP23	Blood (presumed contaminant)	9	+	10^7
RP12	Catheter sepsis	23	+	10^5–10^7
RP62A-NA	Daughter strain of RP62A	21	−	10^5–10^7
PC	Blood from patient with endocarditis	12	−	10^5–10^7
SP22	Blood (presumed contaminant)	24	−	10^5–10^7
SP18	Blood (presumed contaminant)	9	−	10^7
K5	Skin	4	−	10^6
K15	Skin	5	−	10^6
RP39	Blood	10	−	10^7
SP27	Blood	28	−	10^5–10^8

[a] +, Positive reaction; −, negative reaction.

RESULTS

Data were collected from 299 animals in which 13 strains of *S. epidermidis* were used to establish infective endocarditis (Table 1). The numbers of animals studied with each strain varied, dependent in part on whether a particular strain had been used in a previous study examining virulence variability between two species of coagulase-negative staphylococci (2). In the present study, more than 20 rats (range, 21 to 84) were challenged with each of six strains (three slime positive and three slime negative). Each of these six strains was tested at three or four inocula, ranging from 10^4 to 10^9 CFU. Because of premature death in 21 animals, quantitative blood cultures were obtained in 278 rats at the time of sacrifice.

As the concentration of bacteria in blood cultures increased from less than 10 to greater than 100 CFU/ml, the mean bacterial concentrations in vegetations more than doubled (Table 2). In addition, mean weights of vegetations and mean inoculum sizes also increased as bacterial concentrations in blood increased to over 100 CFU/ml. Although no quantitation of catheter cultures was performed, positive catheter cultures also correlated with levels of bacteremia. In particular, catheter cultures were uniformly positive in the 25 rats with the highest level of bacteremia (Table 2).

Significant correlations were demonstrated among blood culture concentrations (CFU/ml) number of organisms per gram of vegetation tissue ($r = 0.34$, $P < 0.001$), and total number of organisms per vegetation ($r = 0.37$, $P < 0.001$) (Fig. 1). Furthermore, blood culture concentrations correlated with vegetation weights ($r = 0.22$, $P < 0.001$), size of infecting inoculum ($r = 0.26$, $P < 0.0001$), and indwelling catheter culture positivity ($r = 0.29$, $P < 0.0001$). These correlations held true for both the 171 rats challenged with slime-positive strains and the 107 rats given slime-negative organisms.

DISCUSSION

Garrison and Freedman (10), using a rabbit model of experimental endocarditis, provided initial animal model data correlating quantitative blood and tissue (not including endocardial) cultures. Rabbits that manifested positive blood cultures at the time of sacrifice generally had much larger concentrations of bacteria in spleen, liver, and kidney tissues as compared with animals with negative blood cultures.

Durack and Beeson (7) subsequently reported blood and endocardial tissue culture quantitation data from 21 experimentally infected rabbits with viridans group streptococcal endocarditis. Bacteria in blood cultures decreased markedly in number (200 to <3 CFU/ml) as animals, sacrificed at various intervals, went from 24 to 72 h postinoculation. In contrast, the concentration of bacteria per gram of vegetation tissue (10^9 CFU/g) remained relatively constant during this time interval.

Using a relatively small number of animals in a rat model of experimental endocarditis, Santoro and Levison (17) noted that blood cultures were routinely positive in 22 catheterized animals more than 6 h postinoculation with a strain of *S. mitis*. Despite vegetation bacterial concentrations varying by more than 1 log ($10^{7.3}$ to $10^{8.4}$ CFU/g), blood cultures usually contained less than 10 CFU/ml. In comparison, blood cultures were positive in only one of three rats at 6 h postinfection, when the mean vegetation concentration of bacteria was somewhat lower ($10^{6.9}$ CFU/g).

The present study examined correlations among blood, catheter, and vegetation specimens obtained at the time of sacrifice of animals infected with strains of *S. epidermidis*. To the best of our knowledge, this is the first time such a detailed examination of the microbiologic characteristics of foreign-body-related endocardial infection has been recorded, regardless of the type of organism used. Interestingly, investigators (M. J. Enzler, M. S. Rouse, N. K. Henry, and W. R. Wilson, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 470, 1985) have recently documented an influence of inoculum size on treatment efficacy in experimental endocarditis due to *S. bovis*. Using the rabbit model of endocarditis, these investigators noticed that the results of therapy, with either procaine penicillin alone, procaine penicillin with streptomycin, or procaine penicillin plus gentamicin, were inoculum dependent. After 24 h of therapy, rabbits infected with the larger inoculum (10^7 CFU) had endocardial vegetations that were larger and contained a greater number of streptococci than did animals challenged with 10^5 CFU of streptococci.

Surface slime production by strains of *S. epidermidis* did not seem to influence the bacterial concentration correlations. Analyses showed that blood, catheter, and vegetation specimens taken from animals infected with any of the five slime-positive strains were very similar in microbiologic characteristics to those taken from rats inoculated with the eight slime-negative strains (data not shown). One might have expected somewhat different results, realizing that prior reports have indicated that slime-negative strains were more easily phagocytosed (2) and that surface slime production interfered with white cell function (11). The increased phagocytosis seen with the slime-negative strains was due entirely to one (PC) of two slime-negative strains tested in in vitro phagocytic killing studies (2). Furthermore, strain PC also accounted for the differences in blood culture positivity rates demonstrated between animals infected with either slime-positive or -negative strains of *S. epidermidis*.

It is difficult to ascertain to what extent colonized, indwell-

TABLE 2. Catheter and vegetation characteristics categorized by level of bacteremia

Blood culture concn (CFU/ml)	Mean (±SD):				% Catheter culture positivity
	No. of organisms/g of vegetation (10^9)	Vegetation wt (mg)	Total no. of organisms/vegetation	Inoculum size ($\log_{10}$)	
0–9 (n = 207)	1.17 ± 7.79	10.4 ± 10.0	(6.24 ± 9.22) × 10^6	6.37 ± 1.14	79.2
10–99 (n = 46)	1.41 ± 1.68	12.8 ± 8.55	(1.36 ± 1.28) × 10^7	6.78 ± 1.07	89.1
≥100 (n = 25)	2.59 ± 7.64	17.2 ± 9.20	(1.59 ± 1.21) × 10^7	6.84 ± 1.21	100

ing catheters influenced bacterial concentrations in blood. Since 83% (229 of 277) of the animals with infected vegetations and blood also had positive catheter cultures, relatively few rats with negative catheter cultures were available for analysis. Negative blood cultures were demonstrated in 30 (62%) of the 48 rats with negative catheter cultures. Although no firm conclusions could be made regarding the direct effect of catheter colonization on bacterial concentrations in blood, evidence from prior studies (8, 9, 12, 13, 15, 16) of experimental endocarditis has confirmed the importance of indwelling catheters in establishing endocardial infection (9), accelerating vegetation bacterial growth (13), maintaining the endocardial infection (8, 16), and interfering with the efficacy of antibiotic prophylaxis (12) and treatment (15). The catheter, therefore, probably is a very important factor, at least indirectly, in influencing the level of bacteremia in the setting of foreign-body-associated infective endocarditis. Studies are ongoing to better define the role of intracardiac catheters on several microbiologic aspects of endocardial infections.

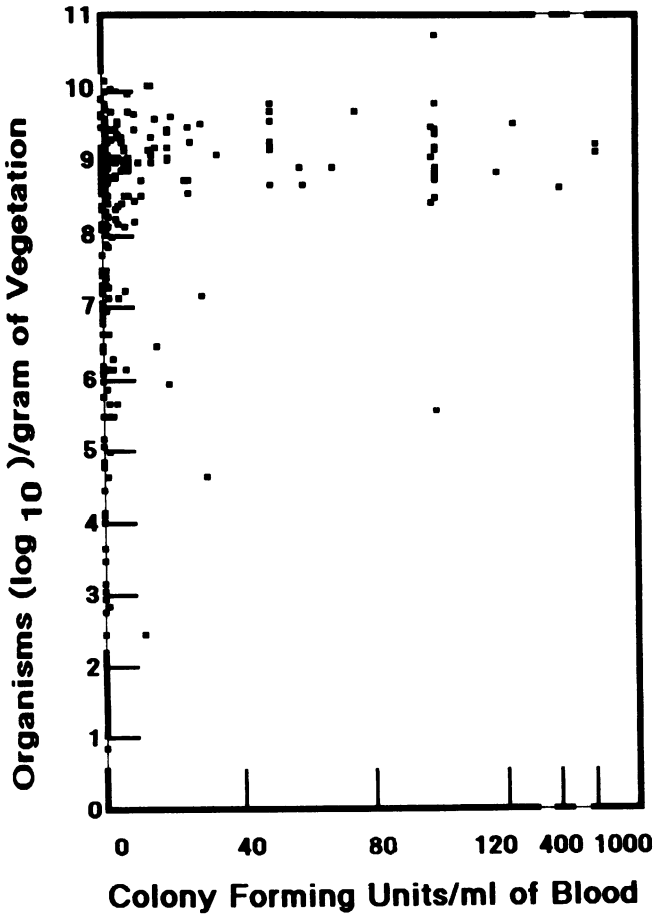

FIG. 1. Correlation of bacterial concentrations in blood and number of organisms per gram of vegetation tissue.

The clinical relevance of examining bacterial concentration correlations is unestablished at this time. Certainly, it would be inappropriate to quantitate blood cultures from patients, in lieu of more established techniques such as two-dimensional echocardiography, to ascertain the sizes of vegetations. Moreover, much variability in concentration correlations may exist for different types of bacteria. At the same time, the data presented here encourage further investigation.

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