Enzyme-Linked Immunosorbent Assay for Detection of *Staphylococcus epidermidis* Antibody in Experimental *S. epidermidis* Endocarditis

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The immune response against *Staphylococcus epidermidis*, as determined by an enzyme-linked immunosorbent assay, was evaluated in experimental *S. epidermidis* infections in rabbits. Antigens from 8 of 10 clinical *S. epidermidis* strains detected significant antibody production in five rabbits immunized with different strains of *S. epidermidis* and in five of six rabbits with experimental endocarditis caused by four different strains. The antigens from two strains detected antibody production in all rabbits, and strain ATCC 14990 discriminated best between positive and negative samples. Consecutive blood samples from rabbits with endocarditis and control rabbits with bacteremia, which were successfully prevented from developing endocarditis by using prophylactic antibiotics, were examined by using an enzyme-linked immunosorbent assay and an ultrasonic extract of strain ATCC 14990 as the antigen. This assay discriminated between rabbits with endocarditis and rabbits with uncomplicated bacteremia. Antibody production was detected as early as 3 days after the onset of infection in rabbits with endocarditis.

*Staphylococcus epidermidis* bacteremia and endocarditis have become important clinical problems (4, 26). Situations which break the normal skin barrier predispose humans to infection with this organism, as *S. epidermidis* is a normal inhabitant of human skin (4). Consequently, *S. epidermidis* infections have become common complications in patients with intravascular cannulae, prosthetic heart valves, and central nervous system shunts (1, 6, 12, 13, 20, 21).

*S. epidermidis* causes 63 to 90% of all cases of prosthetic valve endocarditis (10, 11). Diagnosis generally depends on isolation of coagulase-negative *staphylococci* from blood. However, culture methods and physical examination do not distinguish endocarditis from uncomplicated bacteremia (10), a common occurrence in patients with intravenous or intraarterial cannulae. Isolation of coagulase-negative *staphylococci* from blood may also represent skin contamination, as such organisms are the most common contaminants of blood cultures (16). Furthermore, cultures may be negative in patients with endocarditis who have received prophylactic antibiotics before cardiac valve replacement (17).

Serologic tests have been useful for distinguishing endocarditis from uncomplicated bacteremia in patients with positive blood cultures for *Staphylococcus aureus* (24). In this paper we describe an enzyme-linked immunosorbent assay (ELISA) for detection of antibody to *S. epidermidis* in rabbits with bacteremia caused by coagulase-negative *staphylococci*. Using this assay, we tested the hypothesis that serologic response distinguishes endocarditis from uncomplicated bacteremia caused by *S. epidermidis*.

**MATERIALS AND METHODS**

**Bacteria.** Ten *S. epidermidis* strains were used. Strains A1271/76, A1388/76, A1394/76, and A1389/76 represented the four biotypes of Baird-Parker and were studied previously by one of us (9). All four of these strains were isolated from blood cultures (9); they were obtained from K. Rosendal, Statens Seruminstitut, Copenhagen, Denmark. Two other strains were isolated from patients with bacterial endocarditis. These strains, designated strains A and B, were kindly provided by R. C. Moeller and A. W. Karchmer, Massachusetts General Hospital, Boston, and were isolated from the blood of patients with endocarditis. Another strain, strain C, was obtained from F. D. Lowy, Albert Einstein College of Medicine, New York, N.Y. This strain has been used in the rabbit model of endocarditis (2, 15). Strain ATCC 14990 was obtained from B. Wilkinson, Illinois State University, Normal, and was used as a well-characterized and generally available reference strain. The two remaining strains (strains 60A and IU51) were clinical isolates from the Indiana University Medical Center Hospital, Indianapolis, and had been used in other studies. Strains A, B, C, and IU51 were used in our rabbit model of endocarditis and for preparation of antigens for the ELISA. The other strains were used as vaccines for production of antisera and for production of antigens for use in the ELISA.

**Antigen production.** The bacteria were grown at 37°C on tryptic soy agar (Difco Laboratories, Detroit, Mich.). The bacteria were scraped off the agar, washed four times in saline, adjusted to 20% (vol/vol) of their wet volume following centrifugation at 15,000 × g in saline, and stored at −70°C. Antigen extracts (ultrasonic extracts) were prepared by disrupting a 20% suspension of washed bacteria in distilled water for 4 h in a batch model U20 sonicator (Bronwill Scientific Inc., Rochester, N.Y.). After centrifugation at 15,000 × g for 60 min, the supernatant was stored at −70°C. The protein concentrations of the ultrasonic extracts ranged from 5.3 to 16.5 g/liter, as determined by the method of Lowry et al. (14).

**Immunization of rabbits.** New Zealand White rabbits (2 to 3 kg) were immunized intracutaneously with ultrasonic extracts of *S. epidermidis* strains A1271/76, A1388/76, A1394/76, 60A, and IU51 that were mixed with equal
amounts of complete Freund adjuvant. Rabbits received 2 ml of the vaccine weekly for 5 to 6 weeks and were bled after 2 to 3 months of immunization. Sera were stored at −70°C.

Production of endocarditis. A 17-gauge polyethylene catheter (Desert Co., Salt Lake City, Utah) was placed in the left ventricle or at the aortic valve via the left carotid artery as described previously (18, 23), and 72 h later 2 × 10^8 CFU of S. epidermidis was injected intravenously. Rabbits were sacrificed 9 to 10 days after challenge, and cardiac vegetations were excised, weighed homogenized, and cultured quantitatively. Rabbits with vegetation cultures which contained S. epidermidis were considered to have endocarditis. A total of 38 rabbits were used in this study. The catheter was placed in the left ventricle or at the aortic valve, and vegetations were present in each place. Five rabbits injected with strain IU51 without prior antibiotic prophylaxis developed endocarditis. All other rabbits received intramuscular cefamandole treatment (130 mg/kg) 1 h before challenge, and treatment was continued by using 60-mg/kg doses every 8 h for 72 h (nine doses). Ten rabbits injected with strain IU51, which was resistant to cefamandole, developed endocarditis despite prophylaxis. Two of six rabbits injected with strain A, two of nine rabbits injected with strain B, and two of eight rabbits injected with strain C developed endocarditis. Strains A, B, and C were susceptible to cefamandole. S. epidermidis bacteremia was documented in all rabbits and persisted for 3 to 5 days despite antibiotic administration. Rabbits with negative valve cultures were considered to have uncomplicated bacteremia. A total of 21 rabbits had endocarditis, and 17 had uncomplicated bacteremia. Serum samples were obtained at the time of catheter insertion in 34 rabbits, and on day 1 and day 2 after the onset of infection in 17 rabbits, between day 3 and day 4 in 19 rabbits, and at the time of autopsy in each rabbit. Sera from 10 normal rabbits were used as controls. The sera were stored at −70°C.

ELISA. Polyvinylchloride microelisa plates (type MIC-2000; Dynatech Laboratories, Inc., Alexandria, Va.) were coated with 0.1-ml portions of an ultrasonic extract of S. epidermidis containing 50 μg of protein per ml or 0.2% (vol/vol) whole cells in 0.01 M Tris hydrochloride (pH 7.0). These concentrations were chosen because they gave the greatest difference between sera from rabbits with endocarditis and normal sera. Immediately after application of the cells, an equal volume of 0.25% (wt/vol) glutaraldehyde (Sigma Chemical Co., St. Louis, Mo.) in 0.01 M Tris buffer was added. The following steps were identical for both ELISA systems. After antigens were adsorbed to the wells by incubation for 1 h at 20°C, the wells were aspirated and rinsed. The remaining binding sites were next blocked by incubation with 0.1 ml of 5% (wt/vol) bovine serum albumin (Sigma) in 0.01 M Tris buffer for 1 h at 20°C. After the plates were rinsed, 0.1 ml of test sample diluted 1/200 in bovine serum albumin buffer was added, and the preparation was incubated for 1 h at 20°C. After rinsing, rabbit antibodies bound to the solid-phase antigen were detected by incubation for 1 h at 20°C with an alkaline phosphate-labeled immunoglobulin G fraction of goat antiserum to rabbit immunoglobulin G conjugated as described by Sathapatayavongs et al. (19). The enzyme reaction was stopped adding 0.05 ml of 2 N NaOH to the wells, and the optical density at 405 nm was read by using a Microelisa R Auto Reader (model MR 580; Dynatech Laboratories). Three wells were tested for each sample, and the mean optical density at 405 nm was calculated. On each plate 10 control sera from normal rabbits were tested simultaneously. The results were expressed as a ratio by dividing the optical density at 405 nm of the test sample by the optical density at 405 nm of the 10 normal controls. Ratios which were three times greater than the mean of the controls were considered positive.

RESULTS

Variability in antibody response against different S. epidermidis strains. If serotype or strain specificity exists among S. epidermidis strains, antibody tests performed by using a single strain as the antigen source may not be adequate. To evaluate the heterogeneity of the antibody response, we tested sera from the 11 rabbits immunized or infected with nine strains of S. epidermidis in ELISAs by using ultrasonic extracts and whole cells from 10 S. epidermidis strains as the antigens. The antibody response was homogeneous. Whole cells and ultrasonic extracts from 8 of the 10 strains detected significant antibody production in the five immunized rabbits and in 5 of the 6 rabbits with endocarditis (Table 1). Ultrasonic extracts from two strains, strains IU51 and ATCC 14990, detected significant antibody responses in all 11 rabbits. Strain ATCC 14990 detected antibody production in 11 rabbits, and the ultrasonic extract gave better discrimination between positive and negative samples than whole cells. Thus, an ultrasonic extract from this strain was used in the rest of this study.

Antibody response in S. epidermidis endocarditis and bacteremia. The antibody responses at 7 to 10 days after the onset of infection in rabbits with endocarditis and uncomplicated bacteremia were compared by using an ultrasonic extract of strain ATCC 14990 as the antigen (Fig. 1). The antibody ratios were 4.79 ± 1.93 (mean ± standard deviation) for rabbits with endocarditis, 1.46 ± 0.66 for rabbits with uncomplicated bacteremia, and 0.89 ± 0.35 for normal control rabbits. The mean antibody level in the uncomplicated bacteremia group was significantly higher than the level in the control group (P < 0.01), but no response was positive (i.e., greater than 3.0) (Fig. 1). The antibody response in the endocarditis group was significantly higher than the response in the uncomplicated bacteremia group (P < 0.01), and 19 of 21 samples were positive (Fig. 1). Unsuccessful prophylactic antibiotic administration in rabbits which developed endocarditis diminished the antibody response slightly. The antibody levels in the 16 rabbits which developed endocarditis despite prophylaxis were 4.43 ± 3.05.

| Table 1. Antibody responses in rabbits against different S. epidermidis strains, determined by using whole cells or ultrasonic extracts as the antigens |

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<th>S. epidermidis strain</th>
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<th>Ultrasonic extracts</th>
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<tr>
<td></td>
<td>Immunized</td>
<td>Rabbits with endocarditis</td>
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<td>rabbits (n = 5)</td>
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<tr>
<td>IU51</td>
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<td>ATCC 14990</td>
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* Values are numbers of rabbits positive (ratio of sample mean to control mean ≥ 3.0).
1.65, compared with $5.97 \pm 2.49$ in the five rabbits with endocarditis which did not receive antibiotics ($P < 0.05$).

Figure 2 shows the time course of the antibody responses in the rabbits with endocarditis and uncomplicated bacteremia. The antibody responses discriminated between the two groups as early as 3 to 4 days after challenge (endocarditis, $2.94 \pm 0.98$; transient bacteremia, $1.29 \pm 0.66$; $P < 0.01$). The antibody levels continued to rise in the rabbits with endocarditis but not in the rabbits with uncomplicated bacteremia (Fig. 2).

**DISCUSSION**

Since *S. epidermidis* has not been regarded as a frequent human pathogen, serologic tests for this organism have received minimal attention (3, 22). The antigen heterogeneity of *S. epidermidis* strains would limit the usefulness of serodiagnosis of *S. epidermidis* infections. We found no such heterogeneity when we used ultrasonic extracts of whole staphylococci. A significant antibody response was detected in sera from 10 of 11 rabbits immunized or infected with nine different *S. epidermidis* strains (Table 1). Our findings indicate that there is a high degree of serologic cross-reactivity among *S. epidermidis* strains and that serologic tests performed by using antigenic extracts from one strain are feasible. Previous observations based on quantitative immunoelectrophoretic methods also showed a high degree of homogeneity in antibody responses to *S. epidermidis* (9).

Strain ATCC 14990 was used for our experiments, but we assume that similar results would be obtained if extracts from other strains were used. Ultrasonic extracts from *S. epidermidis* contain at least 43 different antigenic components, including both cytoplasmic and cell wall antigens, such as teichoic acid and peptidoglycan (9). Heterogeneity among individual *S. epidermidis* antigens would probably be masked if whole-organism extracts were used. Since use of purified antigens offered no advantage over whole-organism extracts in serologic tests for antibodies to *S. aureus* (7, 8, 24, 25), we used ultrasonic extracts in this study.

The ELISA performed by using an ultrasonic extract from *S. epidermidis* strain ATCC 14990 appears to be a highly sensitive method for detecting antibodies to *S. epidermidis*. Sera obtained before infection contained only low levels of these antibodies. The levels of antibodies to *S. epidermidis* rose rapidly in rabbits with endocarditis (Fig. 2). As early as 3 to 4 days after the onset of infection immunoglobulin G antibodies appeared in rabbits with endocarditis and distinguished these rabbits from rabbits with uncomplicated bacteremia (Fig. 2). A rapid increase in immunoglobulin G antibody levels was also observed in rabbits with experimental *S. aureus* endocarditis (23).

West et al. (22) recently investigated the antibody response to teichoic acid in experimental *S. epidermidis* endocarditis. Purified teichoic acids from three coagulase-negative staphylococci were used in an ELISA (22). A significant antibody response was first detected 6 days after the onset of endocarditis (22). Control rabbits with uncomplicated bacteremia also developed antibodies to teichoic acids, but the antibody levels discriminated between rabbits with endocarditis and control rabbits at 4 weeks. Sera from rabbits with *S. aureus* endocarditis cross-reacted in assays for antibodies to *S. epidermidis* teichoic acid, and animals with *S. epidermidis* endocarditis developed antibodies to *S. aureus* teichoic acid (22). We have reported similar findings in patients with *S. epidermidis* endocarditis (5). *S. aureus* and *S. epidermidis* are known to share several antigens (9). However, such cross-reactivity should not compromise the use of serologic tests for distinguishing *S. epidermidis* endocarditis from uncomplicated bacteremia. Additional studies are needed to determine whether cross-reactive

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**FIG. 1.** Antibody levels against an ultrasonic extract of *S. epidermidis* strain ATCC 14990, as determined by the ELISA. Ten normal rabbit sera were tested simultaneously, and the mean optical density values were calculated. The results were divided by the mean of the control group, giving a ratio (shown on the ordinate). *N* is the number of rabbits in each group. The horizontal lines indicate group means.
antibodies develop during infections with other microorganisms.

Based on these observations with experimental endocarditis and previously published experience with S. aureus bacteremia (5, 8, 24), serodiagnosis may be useful in patients with S. epidermidis bacteremia. It may be possible to distinguish S. epidermidis endocarditis from uncomplicated bacteremia or contamination of blood cultures in patients with intravascular foreign bodies or prosthetic valves. Using a less sensitive agglutination assay, Bayston (3), found higher levels of S. epidermidis antibodies in children with infected Spitz-Holter shunts than in normal controls.

In conclusion, the ELISA performed by using an ultrasonic extract of S. epidermidis is a highly sensitive method for detecting S. epidermidis antibodies. This assay can distinguish uncomplicated bacteremia from experimental endocarditis in rabbits. These observations suggest that this assay may discriminate endocarditis from uncomplicated S. epidermidis bacteremia in patients with prosthetic valves or other intravascular foreign bodies.

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LITERATURE CITED


