Protection Against Endocarditis Due to
Staphylococcus epidermidis by Immunization
With Capsular Polysaccharide/Adhesin

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Background. Staphylococcus epidermidis is the principal pathogen in prosthetic valve endocarditis. The capsular polysaccharide adhesin (PS/A) has been shown to mediate attachment of bacteria to medical devices. In this study, we investigated the efficacy of active and passive immunization against PS/A in preventing S. epidermidis endocarditis in a rabbit model.

Methods and Results. Aortic valve vegetations were produced by inserting a Teflon catheter into the left ventricle through the right carotid artery. Bacteremia and endocarditis were then established by implanting in the left jugular vein a catheter that was attached to an osmotic pump and contaminated with S. epidermidis strain RP62A. During a 3-week study period, of 64 blood cultures taken every second or third day from six nonimmune rabbits, 54 (84%) yielded strain RP62A. In rabbits actively immunized with PS/A, eight of 60 blood cultures (13%) were positive (odds ratio 5.0, 95% CI, 2.0-12.3, p=0.005). At death, all six nonimmune rabbits had infected vegetations that yielded 10^6-10^11 colony-forming units (cfu)/g of vegetation, whereas only one PS/A-immunized rabbit had an infected vegetation. Immunization protocols designed to elicit antibody to teichoic acid but not to PS/A afforded no protection against bacteremia or endocarditis. Infusion of monoclonal antibody to PS/A through a catheter in the right jugular vein provided a level of protection against both bacteremia and endocarditis comparable to that produced by active immunization. In vitro, antibody against PS/A was opsonic for S. epidermidis.

Conclusions. Immunoprophylaxis targeted at staphylococcal PS/A is a promising new approach to the prevention of prosthetic valve endocarditis. (Circulation 1991;84:2539-2546)

The coagulase-negative staphylococcus, particularly Staphylococcus epidermidis, is a formidable pathogen in patients undergoing prosthetic cardiac valve surgery. Not only is S. epidermidis the principal pathogen in prosthetic valve endocarditis,1-8 but it is also the most frequent cause of intravascular catheter-associated infections in the immediate postoperative period.9-12 Because S. epidermidis is a major component of the normal skin flora and very rarely causes infection in the absence of a foreign body, a number of investigators have hypothesized that this microorganism has a special ability to adhere to, colonize, and infect prosthetic materials because of its unique surface properties. Attention has focused on the ability of some strains, particularly those isolated from infections of medical devices, to produce copious amounts of an extracellular material generally referred to as “slime.”9,13-21 Some investigators believe that slime mediates adherence of staphylococci to prosthetic materials. In addition, it has been noted that slime envelops adherent bacterial colonies in a thick biofilm that may provide protection from host defenses and antibiotics. Despite considerable investigation, however, the precise role of this complex material in the pathogenesis of device-associated coagulase-negative staphylococcal infections remains unclear.

We have previously purified a polysaccharide from slime-producing strains of S. epidermidis that appears to be involved in staphylococcal adherence to plastics, such as silicon elastomer, that are frequently used in catheters and other medical devices.22 In addition to functioning as an adhesin, this polysaccharide has been shown to mediate attachment of bacteria to medical devices.23-25 This adhesin is a capsular polysaccharide from the capsular polysaccharide/adhesin complex of S. epidermidis.25-28 It has been demonstrated that antibodies to this adhesin are protective in a rabbit model of endocarditis.20,29 In addition, it has been shown that immunization results in the development of both opsonic and nonopsonic antibodies to this adhesin.20,29,30 It is possible that the immunization strategy investigated in this study resulted in the development of opsonic antibodies to PS/A that rendered the bacterium more susceptible to opsonic macrophages or that the immunization process altered the adhesin such that it was not recognized by the opsonic antibody.

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charide also serves as a capsule for *S. epidermidis*. Most clinical isolates of *S. epidermidis* were found to produce a serologically identical capsular polysaccharide adhesin (PS/A). Chemically, PS/A is a large (more than 500,000 kD) polymer of neutral sugars rich in galactose and arabinose. In vitro, antibody to purified PS/A inhibited adherence of a number of strains of *S. epidermidis* to silicon elastomer tubing, which suggests that immunoglobulin directed specifically against PS/A might have a role in the prevention of coagulase-negative staphylococcal infections by inhibiting bacterial attachment.

We then extended these in vitro findings to a rabbit model of central venous catheter infection, demonstrating that active immunization with PS/A greatly attenuated bacteremia spawned by catheters that had been contaminated with *S. epidermidis*. Passive infusion of PS/A-specific polyclonal and monoclonal immunoglobulin through a separate catheter placed in the contralateral jugular vein protected the rabbits against both bacteremia and hematogenous colonization of this contralateral catheter. In the present study, we have further explored the potential efficacy of immunoprophylaxis in a rabbit model of endocarditis.

**Methods**

**Bacterial Strains**

*S. epidermidis* strain RP62A is a previously described prototypic slime-producing clinical isolate from which we originally extracted and purified PS/A. *S. epidermidis* strain SE360 expresses teichoic acid (TA) and several surface proteins serologically identical to those of strain RP62A but does not elicit antibody to PS/A in rabbits.

**Antibody Assays and Production of Polyclonal and Monoclonal Antibodies to PS/A**

Capsular PS/A was purified as described previously. To obtain polyclonal antibodies, rabbits were immunized twice weekly for 3 weeks with subcutaneous injections of 100 μg PS/A from strain RP62A in complete Freund’s adjuvant. Antisera were tested for antibody to PS/A and TA by enzyme-linked immunosorbent assay (ELISA) as described previously. Postimmunization sera were tested at dilutions of 1:100 to 1:51,200 and compared to titers in sera obtained just before immunization; postinfection sera were tested at a dilution of 1:100 only, and ELISA readings obtained at this dilution were compared with those obtained using a 1:100 dilution of prechallenge sera. Individual sera were tested in duplicate, and means for groups of similarly treated rabbits represent the means derived from the means of the individual duplicate titers.

Monoclonal antibody was prepared by standard techniques after hyperimmunization of BALB/c mice with 10 μg purified PS/A. One PS/A-specific clone producing an IgG3 subclass antibody and designated IXB2 was used in passive immunoprophylaxis studies. This antibody was obtained from culture supernates of hybridoma cells producing IXB2 antibody after application to a Bakerbond ABx column (J.T. Baker, Phillipsburg, N.J.) and elution with 1 M NaCl and 0.5 M ammonium sulfate. The purified antibody was then dialyzed against phosphate buffered saline.

**Rabbit Model of Catheter-Induced Endocarditis**

New Zealand White rabbits weighing 2.5–3.0 kg were anesthetized with ketamine (Ketalar, 40 mg/kg, Parke-Davis, Morris Plains, N.J.), atropine (100 μg), and xylazine (Rompun, 10 mg/kg, Mobay, Shawnee, Kan.). The neck area was shaved and disinfected with iodine tincture, and a 3-cm incision was made to expose the right carotid artery. A 3F Teflon catheter was inserted into the carotid by cutdown, and the catheter was then passed through the carotid artery into the cavity of the left ventricle (Figure 1). The incision was closed after the tubing was tied in place. This procedure resulted in production of aortic valve leaflet vegetations in all rabbits within 7 days. One week after catheter insertion, rabbits were challenged by insertion of a 2.7F silicon elastomer catheter contaminated by immersion in a suspension of 10⁵ cfu/ml of strain RP62A for 15 minutes. After anesthesia and skin disinfection, a 3-cm incision was made to expose the left jugular vein, and the catheter was inserted 4–5 cm into the vein by cutdown. The external end of the catheter was attached to a subcutaneous osmotic pump (Alzet model 2ML1, Alza Corp., Palo Alto, Calif.) (Figure 1). The pump was filled with 2 ml of 10,000 units/ml heparin, which was delivered continuously for 7 days at 10 μL/hr. This technique had previously been found to result in continuous low-grade bacteremia (less than 10 cfu/ml of blood) for up to 8 days in normal rabbits. Once the heparin in the pump was depleted, bacteremia was no longer detectable. In the endocarditis model, however, sustained bacteremia beyond 8 days was noted, indicating the formation of an independent focus of infection (see below).

**Immunoprophylaxis Regimens**

For active immunization experiments, rabbits were immunized subcutaneously with 100 μg of purified PS/A from strain RP62A in complete Freund’s adjuvant twice a week for 2–3 weeks. This immunization schedule was sufficient to produce a significant increase in anti-PS/A antibody titers by ELISA in all rabbits. Control rabbits were immunized with either adjuvant alone or whole cells of *S. epidermidis* strain SE360, which we have previously shown elicits high titers of antibody to cell wall TA but does not elicit antibody to the PS/A of strain RP62A.

For passive immunotherapy, a dual pump rabbit endocarditis model was used to evaluate the protective efficacy of monoclonal antibody (Figure 1). In these experiments, a second silicon elastomer catheter, which had not been contaminated with *S. epidermidis*, was inserted into the right jugular vein and attached to an osmotic pump filled with 2 ml of either normal rabbit serum or monoclonal antibody IXB2.
Determination of Bacteremia and Endocarditis

Blood cultures were performed just before challenge and every 2–3 days thereafter until the animals were killed. Blood (5 ml) from the ear vein was added to 50 ml of tryptic soy broth containing 0.05% polyanetholsulfonic acid and incubated for up to 10 days at 37°C. Routine subcultures were made on tryptic soy agar. All blood culture isolates were verified as the challenge strain RP62A by colonial morphology, Gram's stain, and biochemical testing. Identification of blood isolates was facilitated by the fact that strain RP62A is alkaline phosphatase-negative. Supernates of positive cultures were checked by Ouchterlony immunodiffusion to ensure that PS/A and TA antigens homologous to the infecting strain were made by the bloodstream isolates.

Twenty to 22 days after bacterial challenge, the rabbits were killed, and cardiac vegetations, the intraventricular Teflon catheter, and the intrajugular silicon elastomer catheters were examined and removed. Rabbits that died before the end of the experimental period were processed immediately in the same way. Vegetations were weighed, homogenized, diluted in saline, and cultured quantitatively on tryptic soy agar. Results were expressed as log cfu/g of vegetation. Catheters were cultured semi-quantitatively by rolling a 1-cm distal segment on tryptic soy agar plates as described by Maki et al. Isolates of *S. epidermidis* were verified as strain RP62A as described above.

Serum Chemistries

Glucose and lipid levels were determined on serum samples submitted to the Tufts University School of Veterinary Medicine Diagnostic Laboratories.

Opsonophagocytosis Assay

Opsonophagocytosis was measured by an assay similar to one described previously. The assay incorporated *S. epidermidis* strain RP62A, human polymorphonuclear leukocytes, rabbit complement adsorbed with 10⁶ cfu/ml of strain RP62A, and serum from either normal rabbits, rabbits immunized with PS/A, or the monoclonal antibody IXB2. White blood cells were prepared from human peripheral venous blood and overlaid on mono/poly resolving medium (Flow Laboratories, Inc, McLean, Va.). Rabbit sera were heat-inactivated at 56°C for 30 minutes. Purified (more than 95%) polymorphonuclear leukocytes (1x10⁶ cells/ml) were suspended in RPMI with 5% fetal calf serum and incubated at 37°C for 90 minutes with adsorbed rabbit complement (1:20 dilution), rabbit serum (1:10 dilution) or monoclonal antibody (2.5/μg/ml), and strain RP62A (6x10⁹ cfu/ml). Viable cfus were determined by counting at the end of this incubation period.

Statistical Analysis

The method of Connolly and Liang was used to adjust estimates of the protection afforded by active and passive immunization. This method was used because of the known interdependence of blood culture results and results from other cultures taken from the same animal. This interdependence violates an assumption of the usual χ² test for binomial proportions. The model uses measurements in which the log of the odds of obtaining a positive blood culture on a given day is considered a linear function of the total number of other days for which a positive blood culture is obtained for that animal. Incorporated into this model are terms for the immune status of the animal and the challenge organism used. With this method we can determine the degree of intranimal dependence by estimating the odds ratio for a positive blood culture for any pair of days from an individual rabbit. This model can also estimate the odds ratio between animals in two groups, taking into account the intra-animal dependence, and thus pro-
vide a more conservative statistical analysis on the protective effect of immunization with PS/A.

Differences in antibody levels between the preinfection and postinfection sera were measured by a paired t test. Opsonophagocytic killing was compared by a Student's t test.

Results

Bacteremia and Endocarditis in Rabbits Challenged With S. epidermidis Strain RP62A

All rabbits in these experiments developed S. epidermidis endocarditis after challenge with the contaminated catheter unless they were protected by active or passive immunization against PS/A. Large infected vegetations were noted uniformly on the aortic valve of nonimmune rabbits (Figure 2), with concentrations of S. epidermidis ranging from $10^4$ to $10^{10}$ cfu/g of vegetation (Figures 4 and 6). Sustained bacteremia occurred in all nonimmune rabbits (Figures 3 and 5). All Teflon catheters inserted through the aortic valve into the left ventricle were found to be coated with a fibrinous sleeve, with 20 to more than 1,000 colonies noted on semiquantitative catheter cultures. Culture-positive vegetations formed on the left ventricular wall opposite the tip of the Teflon catheter in some rabbits, particularly when the catheter tip and myocardium were closely apposed; these vegetations were not cultured quantitatively. Semiquantitative cultures of the contaminated silicon elastomer jugular catheters were still positive when they were removed from the rabbits at the end of the experiments, yielding 380 to more than 1,000 colonies per plate.

Several variations on this experimental protocol were used to determine the parameters necessary for establishing endocarditis. When three rabbits with catheters inserted into the left ventricle were challenged with contaminated jugular catheters attached to osmotic pumps that did not contain heparin, bacteremia and endocarditis did not develop, probably because of a lack of fluid flow through the catheter. Removal of the left ventricular catheter before implantation of the infected catheter–pump combination failed to produce endocarditis, although bacteremia for up to 7 days was noted ($n=3$). When rabbits with left ventricular catheter-induced valvarul vegetations were infected with a bolus dose of $10^6$ cfu/rabbit of S. epidermidis strain RP62A, endocarditis was established, but immune rabbits were not protected. Presumably this very high bacterial inoculum overwhelmed host defenses. Finally, three rabbits with left ventricular catheters that received a sterile catheter–pump combination 1 week later failed to develop bacteremia or endocarditis over the next 3 weeks.

Bacteremia and Endocarditis in Actively Immunized Rabbits

Similar to results previously reported,23 immunization of rabbits with PS/A from strain RP62A induced
TABLE 1. Summary of Blood Culture Results, Actively Immunized Rabbits

<table>
<thead>
<tr>
<th>Immune status</th>
<th>No. of rabbits</th>
<th>Positive blood cultures/total cultures</th>
<th>Percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonimmune</td>
<td>6</td>
<td>54/64 (84%)</td>
<td></td>
</tr>
<tr>
<td>PS/A-immune†</td>
<td>6</td>
<td>8/60 (13%)</td>
<td></td>
</tr>
<tr>
<td>TA-immune‡</td>
<td>3</td>
<td>18/23 (78%)</td>
<td></td>
</tr>
</tbody>
</table>

*Non-immune rabbits immunized with complete Freund's adjuvant. †PS/A-immune rabbits immunized with purified polysaccharide adhesin from S. epidermidis strain RP62A. ‡TA-immune, teichoic acid-immune rabbits immunized with S. epidermidis strain SE360.

A high-titer antibody response to PS/A but not to TA, whereas immunization with S. epidermidis strain SE360 resulted in the production of antibody to TA but not to PS/A (not shown). There was a marked reduction in the percentage of blood cultures that were positive for S. epidermidis strain RP62A during the 20–22-day study period in PS/A-immunized rabbits. Sustained bacteremia was noted in only one of six PS/A-immune rabbits versus nine of nine rabbits in the nonimmune and strain SE360-immune (TA-immune) groups (*p<0.01, Fisher's exact test, Figure 3). Only 13% of all blood cultures were positive in the PS/A-immune rabbits, compared with 84% and 78% in the nonimmune and TA-immune groups, respectively (Table 1). The reason for the death of rabbit 198 could not be ascertained at autopsy.

Examination of the results in Figure 3 indicated that detection of a positive blood culture on a given day could be dependent, in part, on having a previous positive blood culture. Using the method of Connolly and Liang⁹ to take this effect into account, the estimate of the odds ratio for obtaining a positive blood culture comparing TA-immune rabbits with PS/A-immune rabbits was 7.69 (95% CI, 2.8–20.8, *p=0.002). The estimate of the odds ratio for obtaining a positive blood culture comparing nonimmune and PS/A-immune rabbits was 5.00 (95% CI, 2.0–12.3, *p=0.005).

Only one of six rabbits immunized with PS/A had aortic valve vegetations that yielded S. epidermidis on culture (Figure 4). This one rabbit, which also had sustained bacteremia (Figure 3), had 4.0×10⁶ cfu/g of vegetation, and 25 colonies of S. epidermidis were recovered on semiquantitative culture of the left ventricular catheter. In contrast, vegetations obtained from nonimmune and TA-immune rabbits were all infected with very high concentrations of bacteria (Figure 4), and all left ventricular catheters were culture-positive as well.

Contaminated jugular catheters used to challenge the rabbits with S. epidermidis remained culture-positive in all experimental groups, although the number of colonies recovered from these catheters was slightly lower in PS/A-immune rabbits (3.8×10⁸ to 6.1×10⁵ cfu/catheter versus 3.6×10⁵ to more than 10⁶ in the nonimmune and 1.3×10⁶ to 8.4×10² cfu/catheter in the TA-immune group).

**Bacteremia and Endocarditis in Rabbits Passively Immunized With Monoclonal Antibody to PS/A**

Passive infusion of monoclonal antibody to PS/A in the dual pump endocarditis model significantly attenuated bacteremia compared with unprotected rabbits (Figure 5, Table 2). All rabbits receiving normal rabbit serum had sustained bacteremia, and 77% of

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**Figure 5.** Chart showing occurrence of positive (■) and negative (□) blood cultures obtained on postinfection days 1–22 resulting from insertion of catheters contaminated with Staphylococcus epidermidis strain RP62A. Rabbits were passively infused by an osmotic pump attached to a catheter inserted into the right jugular vein containing either normal rabbit serum or monoclonal antibody to PS/A (MAB).

**Figure 6.** Graph showing concentration (cfu/g) of Staphylococcus epidermidis RP62A in aortic valve vegetations of rabbits passively infused with either normal rabbit serum or monoclonal antibody to PS/A (MAB immune). Quantitative values for the three rabbits infused with normal serum that died (see Figure 5) were not obtained, but these vegetations were all found to be colonized with S. epidermidis strain RP62A by culturing the vegetations in tryptic soy broth. All five of the vegetations from rabbits infused with the MAB were sterile by this method.
TABLE 2. Summary of Blood Culture Results, Rabbits Receiving Passive Immunoglobulin Prophylaxis

<table>
<thead>
<tr>
<th>Immune therapy infused</th>
<th>No. of rabbits</th>
<th>Positive blood cultures/total cultures</th>
<th>Percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal serum</td>
<td>6</td>
<td>43/43</td>
<td>79</td>
</tr>
<tr>
<td>MAb to PS/A*</td>
<td>6</td>
<td>10/47</td>
<td>21</td>
</tr>
</tbody>
</table>

*Rabbits infused with monoclonal antibody (MAb) IXB2, specific for capsular polysaccharide adhesin (PS/A) of S. epidermidis strain RP62A.

all blood cultures obtained in the 20–22-day experiment were positive. One of six passively immunized rabbits had sustained bacteremia (although one other rabbit was bacteremic at the time of death on day 3), and 21% of all blood cultures were positive. The odds ratio for obtaining a positive blood culture from a control rabbit versus immunized rabbits was 5.5 (95% CI, 1.6–18.9, p=0.02).

One of the six rabbits given monoclonal antibody had infected aortic valve vegetations (Figure 6); the remaining five rabbits were culture-negative even when nonquantitative broth cultures were used. Rabbits given normal serum infusions were all culture-positive; in three of these rabbits quantitative cultures of vegetables were not obtained, but broth cultures were positive for S. epidermidis strain RP62A. Catheters were not routinely cultured in these experiments.

Other Measures of Infection

As in results previously reported for rabbits with catheter-related bacteremia caused by S. epidermidis,23 the rabbits infected here also developed hypoglycemia and hyperlipidemia concurrent with positive blood cultures. These metabolic changes were noted for infected rabbits throughout the entire 3-week study period and provide an independent confirmation of infection in the rabbits. PS/A immunized or monoclonal antibody–infused rabbits did not develop hypoglycemia or hyperlipidemia unless they also had recurring positive blood cultures.

We have also previously reported that rabbits infected with S. epidermidis make antibodies to the TA antigen as a result of infection. Similar findings were seen in the rabbits that developed endocarditis, in that rabbits actively immunized with adjuvant or infused with normal serum made antibody to TA isolated from strain RP62A (not shown). Significant (p<0.05) increases in titers were observed from 7 days postinfection to the end of the experimental period. Rabbits actively immunized with PS/A or passively infused with PS/A-specific monoclonal antibody did not develop endocarditis or have a significant increase in antibody to TA. Two exceptions were rabbits 165 and 238, which had received an otherwise protective immunotherapy but nonetheless developed bacteremia and endocarditis. These rabbits made immune responses to TA. Rabbits immunized with S. epidermidis strain SE360 had preexisting high levels of antibody to TA and made no further immune response to this antigen after infection (not shown).

In previous studies, rabbits with catheter-related coagulase-negative staphylococcal bacteremia failed to make antibody to PS/A as a result of infection.23 We again found that rabbits actively immunized with adjuvant or S. epidermidis strain SE360 failed to make antibody to PS/A (not shown), despite the development of bacteremia and endocarditis (Figures 3 and 4). In the passive protection study, however, rabbits infused with normal serum made a modest but significant (p<0.05) immune response to PS/A starting 12 days after infection. The reason for this is not clear; this represents the only situation where we have found an immune response to PS/A among infected rabbits.23 Rabbits infused with monoclonal antibody to PS/A, including the infected rabbit, 238, failed to make antibody to PS/A during the 3-week experimental period.

Opsonophagocytic Assays

Killing of S. epidermidis strain RP62A by human polymorphonuclear leukocytes was observed after 90 minutes in the presence of both monoclonal and polyclonal antibody to PS/A from strain RP62A. Comparable polyclonal antibody preparations have been tested previously and found to mediate opsonic killing,23 while the monoclonal antibody IXB2 used in passive therapy of endocarditis has not been tested previously in opsonophagocytosis assays. The monoclonal antibody at a concentration 1/10 of that used for passive protection against endocarditis was significantly more opsonic than normal serum, mediating phagocytic killing of 70.7% (p<0.001) of the inoculum by 90 minutes (not shown). This was comparable to the level achieved by a 1:10 dilution of PS/A-immune polyclonal rabbit serum (65.9% killed). In contrast, in normal rabbit serum the inocula grew to 171% of the initial bacterial concentration during the incubation period.

Discussion

The results of the present study suggest that immunoprophylaxis may provide a reasonable approach to the problem of preventing endocarditis caused by coagulase-negative staphylococci. We have demonstrated that immunization with a surface polysaccharide of S. epidermidis prevents bacterial endocarditis in a rabbit model. Passive infusion of monoclonal antibody to this polysaccharide was equally effective. The precise mechanism for antibody-mediated protection remains to be resolved. It is tempting to speculate that antibody to the polysaccharide, whether actively or passively acquired, interrupts the first step in the pathogenesis of infection—attachment of the staphylococcus to the foreign body. Indeed, monoclonal antibody inhibits adherence of S. epidermidis to silicon elastomer and other plastics in vitro (unpublished data). Although we have not tested the role of staphylococcal PS/A in mediating adherence to all of the specific materials used in
prosthetic valve surgery, considerable clinical experience suggests that S. epidermidis avidly colonizes and infects a wide range of prosthetic materials of various compositions.16,13,15,30–32

On the other hand, it is possible that antibody to S. epidermidis polysaccharide protects against infection by a more conventional immune mechanism, opsonophagocytosis of bacteria in blood. In addition to functioning as an adhesin, PS/A is an important component of the capsule of S. epidermidis. We found that antibody to PS/A promoted opsonophagocytic killing of S. epidermidis in the presence of polymorphonuclear leukocytes, as would be expected for an antibody raised against a bacterial capsular antigen.

We have found serologically identical polysaccharide on the surface of almost all clinical isolates of S. epidermidis we have examined and have documented opsonic activity of antibody raised against strain RP62A PS/A for several other staphylococcal strains. Thus, these immunological principles should be generally applicable to the prevention of S. epidermidis prosthetic valve endocarditis.

The rabbit model of endocarditis used in these experiments differs from rabbit and rodent models used by previous investigators in two respects.33–35 First, the bacterial challenge occurred 7 days after insertion of the left ventricular catheter rather than after a 24- to 48-hour interval. More importantly, a relatively low, persistent bacterial challenge was delivered by a contaminated catheter in the jugular vein rather than by one-time injection of a very high bacterial inoculum through a peripheral vein. Our method was found to produce endocarditis very reliably while exposing the left ventricular catheter and aortic valve to levels of organisms more likely to be encountered in clinical practice. Indeed, in order to establish coagulase-negative staphylococcal endocarditis by bolus injection of microorganisms, a dose of 10^9 cfu/rabbit was needed, clearly well above any level of exposure a patient might receive. Not surprisingly, this level of challenge dose overwhelmed host defenses when we tried to protect rabbits by immunization with PS/A. We also found that endocarditis was not produced if the catheter in the aortic valve was removed before bacterial challenge (data not shown). Thus, it seems likely that infection of the catheter itself occurs initially, followed by infection of the adjacent valve leaflets. Therefore, this model may mimic some aspects of prosthetic valve endocarditis because of the need for a foreign body in the heart in order to establish infection by coagulase-negative staphylococci.

Native heart valves are relatively resistant to infection with coagulase-negative staphylococci. Although coagulase-negative staphylococcal endocarditis clearly can occur on damaged native valves,36–38 such infections are uncommon. In contrast, prosthetic heart valves of all types are prone to infection with coagulase-negative staphylococci, especially S. epidermidis, which is the most frequent cause of prosthetic valve endocarditis.1–8 The vast majority of cases of S. epidermidis endocarditis that occur in the 12-month period following surgery appear to be nosocomial in origin.6,7,30 Inoculation of staphylococci probably occurs most frequently in the operating room. The principal source of S. epidermidis is the patient’s own skin flora, but carriers of S. epidermidis on the surgical staff39,40 and contaminated cardiopulmonary bypass blood41 have also been implicated by careful typing of bacterial isolates. Some valves may be seeded as a result of intravascular catheter-associated bacteremia, which occurs frequently in the immediate postoperative period.

Regardless of the specific source of the microorganisms that infect prosthetic valves, S. epidermidis endocarditis is very difficult to treat. Infection frequently involves the valve ring and adjacent myocardium, leading to dehiscence or valve dysfunction and conduction abnormalities.7 In addition, the majority of strains are methicillin-resistant, which considerably complicates antibiotic therapy.7 Not surprisingly, surgical removal of the infected valve is ultimately required in most cases.7,30 Thus, prevention of infection is of paramount importance. Unfortunately, the usual cephalosporin regimens used for antibiotic prophylaxis are ineffective against methicillin-resistant strains of staphylococci. Resistance to vancomycin, the most commonly used alternative antibiotic, has already been reported.42 Inoculation of the heart valve might be avoided in many patients by scrupulous attention to surgical aseptic technique, but even the most experienced surgical teams have been unable to prevent this complication entirely.

In conclusion, immunoprophylaxis targeted at the capsular PS/A of S. epidermidis may provide a promising new approach to the control of staphylococcal infections of prosthetic heart valves and other implantable medical devices.

References

KEY WORDS • coagulase-negative Staphylococcus • prostheses • immunity
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