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Antibodies to Capsular Polysaccharides Are Not Protective against Experimental *Staphylococcus aureus* Endocarditis

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The protective efficacy of antibodies to the *Staphylococcus aureus* capsular polysaccharide was examined in a rat model of catheter-induced endocarditis. Capsular antibodies were induced either by active immunization with killed *S. aureus* or by passive immunization with hyperimmune rabbit antiserum to *S. aureus*. Control rats were injected with phosphate-buffered saline or passively immunized with normal rabbit serum or rabbit antiserum to a nonencapsulated strain. Animals with indwelling catheters were challenged intravenously with 5×10^4 to 4×10^6 CFU of the homologous *S. aureus* strain (capsular serotype 5 strain Reynolds or serotype 1 strain SA1 mucoid). Both immunized and control rats developed *S. aureus* endocarditis. The numbers of *S. aureus* cells recovered from the blood and aortic valve vegetations of immunized rats were similar to those of control rats, indicating that capsule-specific antibodies were not protective. To determine whether the presence of an indwelling catheter interfered with antibody-mediated protection against *S. aureus* endocarditis, catheters were removed 2 h after insertion in additional groups of rats. An inoculum of 10^8 CFU of strain Reynolds was needed to provoke endocarditis in rats catheterized for 2 h, compared with 5×10^4 CFU for rats with indwelling catheters. Passively transferred capsular antibodies were not protective since both immunized and nonimmunized animals developed endocarditis, and quantitative cultures of blood and valvular vegetations revealed no differences between immunized and control animals. The findings of this study indicate that antibodies to the capsular polysaccharide are not protective in the rat model of experimental *S. aureus* endocarditis.

Although there appears to be little resistance to mucosal colonization by staphylococci, healthy humans and animals possess a high degree of innate resistance to *Staphylococcus aureus* infections. This natural immunity is attributed to epidermal and mucosal surface barriers as well as to intact cellular and humoral immune defenses. Nonetheless, this organism remains an important pathogen in individuals with compromised host defenses, causing infections such as bacteremia, endocarditis, arthritis, and osteomyelitis (31, 37). Once *S. aureus* has penetrated the skin or mucosal barriers, host defense is largely dependent upon phagocytic clearance of the bacterium by polymorphonuclear leukocytes. Antibodies to surface antigens of *S. aureus*, including capsular polysaccharides, protein A, and teichoic acid, are opsonic in an in vitro assay (20, 24, 45). However, antibodies to protein A and teichoic acid are not protective in animal models of *S. aureus* infection (20, 21).

Capsular polysaccharides are elaborated by more than 90% of clinical isolates of *S. aureus*; 11 serotypes of encapsulated strains have been reported (23, 39). Strains of serotypes 1 and 2 are heavily encapsulated, easily distinguished by their mucoid colonial morphology, and yet rarely encountered clinically. Antibodies to heavily encapsulated strains are opsonic in vitro and have been shown to confer protection in animal models of infection (12, 15, 28). *S. aureus* strains belonging to the remaining serotypes form nonmucoid colonies on agar plates and are referred to as microencapsulated (44). Approximately 75% of *S. aureus* clinical isolates produce either serotype 5 or serotype 8 capsules (17, 22, 39). Naturally occurring antibodies to these capsular polysaccharides are detectable in normal human serum (3, 8), but the role that these antibodies play in immunity to staphylococcal infections has not been investigated.

The purpose of this study was to examine the protective efficacy of antibodies to the *S. aureus* capsular polysaccharide in invasive staphylococcal disease. Our hypothesis was that capsular antibodies might augment bacterial clearance from the bloodstream of infected animals by enhancing opsonophagocytic killing. To this end, an experimental model of catheter-induced endocarditis was used in rats infected with serotype 1 or 5 strains of *S. aureus.*

MATERIALS AND METHODS

Bacteria. The *S. aureus* strains used in this study are listed in Table 1. For optimal capsule expression, staphylococci were grown overnight at 37°C on Columbia agar (Difco Laboratories, Detroit, Mich.) supplemented with 2% NaCl (29). Colonies were scraped from the agar surface and suspended in 10 mM sodium phosphate–0.15 M NaCl (pH 7.3; phosphate-buffered saline [PBS]) to an optical density at 650 nm of 0.43. Bacteria were diluted to the appropriate concentration, and CFU were verified by plate counts performed in duplicate on tryptic soy agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.).

Immunizations. Male Sprague-Dawley rats were obtained from Charles River Laboratories, Wilmington, Mass. They weighed 195 to 206 g upon arrival, were housed three or four per cage, and were given food and water ad libitum.

Five different immunization protocols were used to determine whether capsular antibodies were protective in the rat model of catheter-induced *S. aureus* endocarditis. In two experiments, the rats were actively immunized; in three subsequent experiments, the rats were passively immunized with rabbit antiserum.

In the first study, 17 rats were injected subcutaneously on days 0, 5, and 10 with PBS or 3×10^8 CFU of formalin-killed strain Reynolds. The animals were challenged with 5×10^4 CFU of strain Reynolds 7 days after the third immunization. In the second study, nine rats were hyperimmunized by injection with 3×10^8 CFU of formalinized strain Reynolds three times per week for 2 weeks and once per week for the following 2 weeks. The animals were rested for 24 days, given a booster dose, and challenged with 2×10^6 CFU of strain Reynolds 8 days later. All rats were bled before each immunization so that serum antibody titers could be evaluated.

Rats in the subsequent three experiments were passively immunized with hyperimmune rabbit antiserum, preimmune (normal) rabbit serum (NRS), or PBS. Hyperimmune rabbit serum was obtained from New Zealand White rabbits

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TABLE 1. *S. aureus* strains used in this study

Strain	Relevant properties	Reference
Reynolds	$CP5^+$	23
JL240	$CP5^-$, EMS mutagenesis[a]	2
SA1 mucoid	$CP1^+$	25
JL25	$CP1^-$, Tn*551*	25
NT857	Nontypeable	2

[a] EMS, ethyl methanesulfonate.

(Millbrook Farms, Amherst, Mass.) by immunizing them intravenously with heat-killed strain Reynolds, formalinized strain NT857, or formalinized strain SA1 mucoid, as described previously (45). The capsular antibody responses elicited by heat- and formalin-killed bacteria are similar, and both sera are opsonic in vitro (45). All sera were heat inactivated at 56°C for 30 min. To remove antibodies to teichoic acid and peptidoglycan, NRS and immune rabbit sera raised to encapsulated strains were adsorbed with trypsinized, nonencapsulated *S. aureus* (strain JL240 or JL25) as described previously (28). Recipient rats were bled before passive immunization and again before bacterial challenge for assessment of serum antibody titers.

In the first passive immunization study, 38 rats with indwelling catheters were injected intraperitoneally with 1 ml of PBS, NRS, or antiserum to strain Reynolds or strain NT857. Twenty-four hours later, they were challenged with 5×10^4 CFU of strain Reynolds. The second passive immunization study involved 18 rats catheterized for only 2 h to assess the effects of foreign body removal on antibody-mediated protection. Two to 4 h after removal of the catheters, the rats were injected with 1 ml of PBS, NRS, or antiserum to strain Reynolds. Twenty-four hours later, the rats were challenged intravenously with 10^8 CFU of strain Reynolds. The final experiment involved passive immunization of 35 rats that had indwelling catheters with antiserum to the serotype 1 strain SA1 mucoid; 24 h later, the animals were challenged intravenously with 4×10^6 CFU of strain SA1 mucoid.

ELISA. Serum antibodies to capsular polysaccharide type 1 (CP1), capsular polysaccharide type 5 (CP5), and teichoic acid were measured by an indirect enzyme-linked immunosorbent assay (ELISA) (26) with the following modifications. The purified polysaccharides were coupled to poly-L-lysine (Sigma Chemical Co., St. Louis, Mo.) as described by Gray (19) and adsorbed to the surface of wells of Immulon II microtiter plates (Dynatech Laboratories, Chantilly, Va.) at a final concentration of 2 μg/ml for CP1 and 4 μg/ml for CP5. Antibodies to teichoic acid were measured on plates coated with this antigen (1 μg/ml) without coupling to poly-L-lysine. Serum from each animal was diluted 100-fold in PBS containing 0.05% Tween 20 (pH 7.4; Sigma) and then serially diluted 2- or 10-fold in the same buffer. Alkaline phosphatase-conjugated antiserum specific for either rat or rabbit immunoglobulin G (IgG; whole molecule) or for rat IgM (μ-chain specific) was obtained from Organon-Teknika Corporation, West Chester, Pa.; *p*-nitrophenyl phosphate substrate was obtained from Kirkegaard & Perry Laboratories, Gaithersburg, Md. Antibody titers were expressed as the reciprocal of the highest serum dilution with an A_{405} of ≥0.35 30 min after addition of the substrate.

Opsonophagocytic assay. The opsonic activity of rabbit and rat sera was determined as described by Xu et al. (45) with the following modifications. Serum samples were tested undiluted or diluted 10-fold in minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) containing 1% bovine serum albumin and then serially diluted 2-fold in the same medium. The opsonic antibody titer was calculated as the reciprocal of the highest serum dilution that killed ≥50% of the strain Reynolds inoculum.

Catheter-induced endocarditis. The rat model of catheter-induced endocarditis described by Baddour et al. (5) was used for this study. In brief, rats were anesthetized with a mixture of ketamine (35 mg/kg; Aveco Co., Fort Dodge, Iowa), xylazine (10 mg/kg; Miles Inc., Shawnee Mission, Kans.), and atropine (0.09 mg/kg; Anpro Pharmaceutical, Arcadia, Calif.). A polyethylene catheter (Intramedic PE10; Clay-Adams, Parsippany, N.J.) was passed through the right carotid artery and aortic valve into the left ventricle. Vigorous pulsation of blood within the catheter indicated correct positioning of the device. The catheter was sealed and tied in place with sterile suturing material, and the incision was closed.

Rats with indwelling catheters were rested for 2 days before tail vein injection with 200 μl of *S. aureus* cells. Within 1 h of challenge, heparinized blood was collected from each animal by tail vein puncture. Quantitative blood cultures were performed by plating dilutions of each sample on tryptic soy agar plates containing 5% sheep erythrocytes (Becton Dickinson). Four days after bacterial challenge, the rats were sacrificed, and their hearts were removed. The aorta and left ventricle were opened, and the vegetations were excised, weighed, and homogenized. Serial dilutions of the homogenates were quantitatively cultured, and the number of CFU of *S. aureus* per gram of vegetation was calculated. For blood samples yielding no growth on agar media, the bacterial concentration was considered to be the lower limit of detection (10 CFU/ml). For animals with sterile or no vegetations, the bacterial concentration was estimated at 1.3×10^3 CFU/g, the minimum level detectable by the culture methods used.

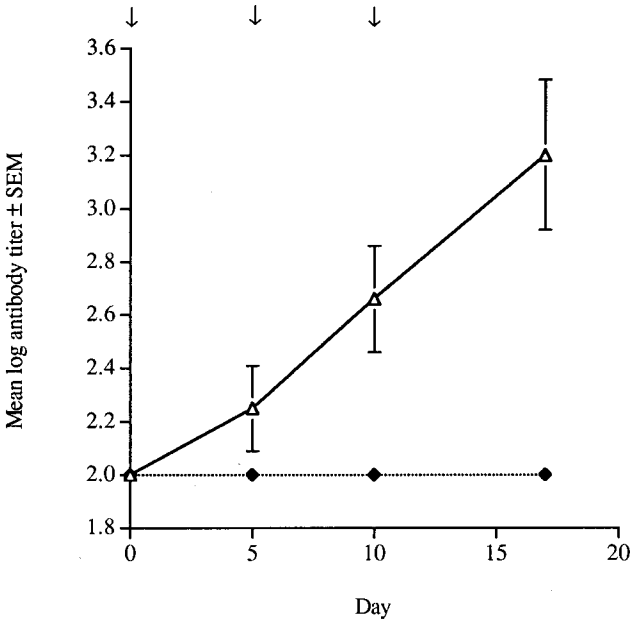

FIG. 1. Comparison of geometric mean serum IgG titers to CP5 in rats immunized on days 0, 5, and 10 (↓) with formalin-killed *S. aureus* Reynolds (△) ($n = 6$) or injected with PBS (◆) ($n = 11$). SEM, standard error of the mean.

Colony immunoblot. The stability of CP5 expression by *S. aureus* Reynolds was measured by immunoblot testing of colonies plated directly from infected vegetations (27). Approximately 600 colonies from immune animals and 600 colonies from nonimmune animals were examined serologically for capsule production with either polyclonal or monoclonal antibodies specific for CP5 (30).

Statistical analysis. The data were analyzed by the unpaired Student *t* test (38) or a binomial test for proportions (16).

RESULTS

Active immunization studies with strain Reynolds. Rodents respond poorly to immunization with purified capsular polysaccharides (14, 28), but they produce high levels of capsular antibodies when immunized with killed encapsulated *S. aureus* cells (28). We injected 17 rats with PBS or formalin-killed *S. aureus* Reynolds and measured their levels of antibody to CP5 by ELISA. Rats immunized with strain Reynolds had higher levels of CP5 antibodies than rats given PBS (Fig. 1). Geometric mean antibody titers to CP5 measured 1 h before bacterial challenge were 1,260 and <100 for immune and nonimmune animals, respectively (Table 2). When a separate group of rats was given a booster dose of killed *S. aureus* 3 weeks after completion of the normal immunization schedule, CP5 antibody titers did not increase further (data not shown). Sera collected and pooled on day 17 from rats actively immunized with strain Reynolds or injected with PBS showed opsonic titers of 20 and <1, respectively.

To determine whether the induction of capsular antibodies correlated with protection, the immunized animals were catheterized and challenged intravenously with 5×10^4 CFU of strain Reynolds. The data in Table 2 indicate that CP5 antibodies were not protective against staphylococcal endocarditis. Numbers of *S. aureus* cells recovered from the blood and aortic valve vegetations were similar whether rats were given PBS or actively immunized with strain Reynolds.

Colonies of strain Reynolds cultivated directly from colonized valvular vegetations of immune and nonimmune animals

TABLE 2. Effects of active immunization on rats challenged with 5×10^4 CFU of *S. aureus* Reynolds

Treatment	Geometric mean anti-CP5 IgG ELISA titer (range)	Opsonic antibody titer	Log CFU/ml of blood 1 h postinoculation (mean ± SEM)	No. of rats with colonized vegetations/ total no. (%)	Log CFU/g of vegetation (mean ± SEM)
PBS	<100	<1	1.1 ± 0.1	8/11 (73)	7.2 ± 1.2
Strain Reynolds	1,260 (200–12,800)	20	1.3 ± 0.2	4/6 (67)	6.7 ± 1.8

uniformly expressed CP5 detectable by colony immunoblot. This finding corroborates that of a previous study (7) indicating that production of the microcapsule is stably maintained during in vivo growth of strain Reynolds.

To increase capsular antibody titers, we hyperimmunized additional groups of rats with killed cells of strain Reynolds (Table 3). These animals responded with antibodies to CP5, but titers decreased after the initial 2 weeks of the immunization period. Booster doses of *S. aureus* given on days 18, 25, and 49 after the initial dose did not stimulate the antibody response (data not shown). At the time of bacterial challenge (8 weeks after the initial vaccine dose), the geometric mean CP5 antibody titer (340) was lower than that (1,260) of animals given only three doses. ELISA data obtained with a μ-chain-specific antibody conjugate indicated that the CP5 antibodies produced in response to active immunization with killed *S. aureus* cells were primarily of the IgM class (data not shown). The hyperimmunized rats were challenged with a larger dose of strain Reynolds (1.6×10^6 CFU) than that used for the immunized rats since each rat weighed approximately 500 g following completion of the hyperimmunization protocol. Eight of nine hyperimmunized animals developed endocardial vegetations, and their cultures yielded 10.7 to 11.6 log CFU of strain Reynolds per g of vegetation (Table 3). No apparent relationship between CP5 antibody level and immunity to endocarditis was observed.

Passive immunization studies with antiserum to strain Reynolds. Because active immunization with killed *S. aureus* elicited a variable CP5 antibody response, we passively immunized 38 additional rats with high-titered rabbit antiserum to strain Reynolds (adsorbed with a nonencapsulated mutant, JL240). The rabbit antiserum had a CP5 antibody titer of 10^6 (measured by ELISA), and it promoted opsonophagocytic killing of strain Reynolds (titer, 1,280).

Before immunization, all rats had ELISA antibody titers of <100 to both CP5 and teichoic acid. Mean ELISA antibody titers in the sera of recipient rats passively immunized with adsorbed Reynolds antiserum were 10,000 against CP5 and <100 against teichoic acid. In the opsonophagocytic killing assay, the titer of pooled serum from rats injected with strain Reynolds antiserum was 20, whereas that of animals given NRS or PBS was <1. Rats that received NT857 antiserum had mean ELISA antibody titers of <100 and 10,000 against CP5 and teichoic acid, respectively, whereas animals injected with NRS or PBS had antibody titers of <100 against both antigens. When challenged with strain Reynolds, most of the animals in all four groups developed endocarditis (Table 4). Quantitative cultures of the infected vegetations from animals in each group were similar, ranging from 8.0 to 10.9 log CFU of *S. aureus* per g of tissue. Thus, neither antibody to CP5 nor antibody to teichoic acid was protective against endocarditis.

In a subsequent experiment, we examined whether the presence of indwelling catheters in the rats interfered with antibody-mediated protection. A preliminary study was conducted to determine the effects of catheterization time and bacterial inoculum on the induction of endocarditis. As shown in Table 5, an inoculum of $\geq 10^8$ CFU of strain Reynolds was needed to provoke endocarditis in the majority of rats catheterized for only 1 h. When we compared catheterization times of 1, 2, and 24 h, we observed no significant differences in infection rates among the three groups of rats. We chose a 2-h catheterization period and an inoculum of 10^8 CFU of strain Reynolds for the subsequent passive immunization experiments.

Eighteen rats were catheterized for 2 h. Two to four hours after the catheters were removed, the rats were injected intraperitoneally with strain Reynolds antiserum, NRS, or PBS. The following day, the rats were challenged intravenously with 10^8 CFU of *S. aureus* Reynolds. Twenty hours after challenge, rats passively immunized with Reynolds antiserum had bacterial counts in the blood similar to those of control rats (Table 6). Likewise, quantitative cultures of endocardial vegetations were similar for immune and nonimmune rats. Thus, passive immunization did not protect rats from staphylococcal endocarditis in the absence of a foreign body.

Passive immunization with antiserum to strain SA1 mucoid. SA1 mucoid is a heavily encapsulated serotype 1 strain of *S. aureus* that is virulent in animal models of staphylococcal infection (25, 26). Specific antibodies to CP1 protect mice against bacteremia and renal abscess formation provoked by this strain (28). To determine whether antibodies to CP1 would protect rats against catheter-induced endocarditis, we passively immunized rats with strain SA1 mucoid rabbit antiserum that

TABLE 3. Effects of hyperimmunization on rats challenged with 2×10^6 CFU of *S. aureus* Reynolds

Rat	Anti-CP5 IgG ELISA titer[a]	Log CFU/ml of blood 60 min postinoculation	Log CFU/g of vegetation
1	1,600	2.0	11.6
2	800	1.6	10.7
3	800	<1.5	<3.2
4	400	1.1	11.2
5	400	1.6	11.0
6	200	2.2	11.2
7	200	1.9	10.7
8	100	1.6	11.2
9	<100	2.1	11.0

[a] Geometric mean serum antibody titer of 340.

TABLE 4. Effects of passive immunization on rats with indwelling catheters challenged with 5×10^4 CFU of *S. aureus* Reynolds

Treatment[a]	No. of rats with colonized vegetations/ total no. (%)	Log CFU/g of vegetation (mean ± SEM)
PBS	3/3 (100)	8.0 ± 2.6
NRS	13/15 (87)	9.0 ± 1.0
NT857 antiserum[b]	6/6 (100)	10.9 ± 0.1
Reynolds antiserum	12/14 (86)	8.0 ± 0.9

[a] Rats were injected intraperitoneally with 1 ml of rabbit serum adsorbed with trypsinized strain JL240 or with PBS.
[b] NT857 antiserum was not adsorbed.

TABLE 5. Effects of variation in inoculum and time of catheterization on the ability of *S. aureus* Reynolds to elicit endocarditis in rats

Time of catheterization (h)	Log CFU of challenge dose/rat	No. of rats with colonized vegetations/ total no. (%)	Log CFU/g of vegetation (mean ± SEM)
1	6.0	0/3 (0)	<3.1 ± 0.0
1	7.0	1/4 (25)	5.1 ± 2.3
1	8.0	2/3 (67)	8.4 ± 3.2
2	7.9	3/4 (75)	8.2 ± 2.1
24	7.9	4/5 (80)	8.8 ± 1.7

TABLE 6. Effects of passive immunization on rats challenged with 10^8 CFU of *S. aureus* Reynolds (catheter removed 2 h after placement)

Treatment[a]	Log CFU/ml of blood at 20 h postinoculation (mean ± SEM)	No. of rats with colonized vegetations/ total no. (%)	Log CFU/g of vegetation (mean ± SEM)
PBS	3.2 ± 0.2	8/10 (80)	8.6 ± 1.3
NRS	2.8 ± 0.3	5/11 (45)	6.7 ± 1.2
Reynolds antiserum	2.6 ± 0.2	5/12 (42)	6.3 ± 1.1

[a] Rats were injected intraperitoneally with 1 ml of PBS or rabbit serum adsorbed with trypsinized strain JL240.

was adsorbed with a nonencapsulated mutant, JL25. Before bacterial challenge, rats injected with immune serum had mean anti-CP1 titers of 10,000, whereas rats given adsorbed NRS or PBS had titers of <100. Titers of antibody to teichoic acid were <100 in all rats.

The strain SA1 mucoid inoculum required to induce endocarditis in 50% of catheterized rats was $\sim 4 \times 10^6$, a dose 1,000-fold higher than that reported for strain Reynolds (7). This result is consistent with the observation in a previous study that the capsule attenuates virulence in the rat model of endocarditis (7). Antibodies to CP1 marginally enhanced bacterial clearance from the blood of infected rats. As shown in Table 7, animals passively immunized with antiserum to SA1 mucoid had fewer bacteria per milliliter of blood 40 min after inoculation than did rats given PBS ($P = 0.01$). However, differences between the levels of bacteremia in rats given immune serum and that in rats given preimmune serum were only significant at a P of 0.08. Antibodies to CP1 did not protect rats against experimental endocarditis with strain SA1 mucoid. Approximately 50% of the rats in each group developed endocarditis, and quantitative cultures of endocardial vegetations revealed no differences between immune and nonimmune animals.

Although the highly encapsulated phenotype of strain SA1 mucoid is stable in vitro (25), CP1 expression proved to be less stable in vivo in the endocarditis model. Bacterial colonies isolated from the blood early in the infection (~40 min after challenge) were uniformly mucoid. However, many of the colonies cultured from vegetations on day 4 were not mucoid, even though they were otherwise phenotypically identical to colonies of strain SA1 mucoid. Capsular antibodies did not influence mucoidy; the mean proportions of colonies ± standard errors of the means that reverted to a nonmucoid phenotype were 31.5 ± 15.0 and 45.3 ± 11.9 among immune and nonimmune animals, respectively ($P = 0.47$).

DISCUSSION

The results of our investigation indicate that antibodies to *S. aureus* capsular polysaccharides are not protective against experimental endocarditis. The staphylococcal capsule is unlikely to be the bacterial adhesin responsible for attachment to the damaged cardiac valve. We postulated that capsular antibodies might protect against endocarditis by promoting opsonophagocytosis, resulting in enhanced blood clearance of the bacterial cells. Such protection was achieved in a mouse model of serotype 1 *S. aureus*-induced bacteremia and renal abscess formation (28). Although serum antibodies to the capsule are opsonic in vitro (24, 45), results from this study suggest that the antibodies do not enhance bacterial clearance from the blood of rats or prevent the animals from developing endocarditis.

We chose the rat model of catheter-induced endocarditis to evaluate immunity induced by *S. aureus* capsular antigens for several reasons. The pathogenesis of *S. aureus* endocarditis in animals is similar to that in humans (6), and the *S. aureus* inoculum needed to provoke endocarditis is relatively low ($\sim 10^4$ CFU per rat). In addition, antibody-mediated protection against the development of experimental infective endocarditis has been demonstrated for other microbial pathogens, including streptococci (1, 11, 43), *Staphylococcus epidermidis* (41), *Pseudomonas aeruginosa* (4), and *Candida albicans* (34).

Two previous studies have examined the role of antibodies to staphylococcal surface antigens in protection against experimental endocarditis. In the first, Schennings et al. (35) reported that immunization with a recombinant fibronectin-binding protein provided a modest degree of protection against *S. aureus* endocarditis. In their study, 7 of 11 immunized rats and 10 of 11 nonimmunized control animals developed endocarditis. Fewer *S. aureus* cells were recovered from the valvular vegetations of immunized rats than from those of nonimmune rats 1.5 days after bacterial challenge. Perhaps evaluation of animals earlier than 4 days after infection provides a more sensitive measure of protection than does later assessment. In the second study, Greenberg et al. (21) showed that antibodies to killed *S. aureus* did not protect rabbits against staphylococcal endocarditis. The *S. aureus* strains tested included a nonencapsulated strain and a methicillin-resistant isolate with an undetermined capsular phenotype. Since most blood isolates of *S. aureus* are encapsulated (39), additional studies assessing efficacy of capsular antibodies in experimental endocarditis seemed warranted.

Baddour et al. (7) reported that the *S. aureus* microcapsule attenuates virulence in the endocarditis model. Consistent with their study, we found that the inoculum of the highly encapsulated strain SA1 mucoid required to induce endocarditis in 50% of the rats was at least 1,000-fold greater than that of the microencapsulated strain Reynolds (7). The observation that antibodies to CP1 were not protective was unexpected because previous studies of mice had demonstrated that CP1-specific antibodies enhanced blood clearance of a serotype 1 strain and protected the animals against renal abscess formation (28).

TABLE 7. Effects of passive immunization on rats challenged with 4×10^6 CFU of *S. aureus* SA1 mucoid

Treatment	Log CFU/ml of blood at 40 min postinoculation (mean ± SEM)	No. of rats with colonized vegetations/ total no. (%)	Log CFU/g of vegetation (mean ± SEM)
PBS	2.9 ± 0.2	5/11 (45)	6.0 ± 1.1
NRS	2.6 ± 0.1	6/13 (46)	6.3 ± 1.0
Anti-SA1 mucoid	2.2 ± 0.2	6/11 (55)	6.3 ± 1.0

The reversion of strain SA1 mucoid to a nonmucoid phenotype during endocardial infection was unique to this strain and suggests that loss of capsule expression may confer a selective advantage to this organism in vivo. Perhaps the capsule expressed by strain SA1 mucoid masks bacterial adhesins for fibronectin, platelets, or fibrin on the damaged endothelial surface. Encapsulated strains of group B streptococci (42), *Streptococcus pneumoniae* (36), *Neisseria meningitidis* (9), and *Haemophilus influenzae* type b (40) have been shown to be less adherent to epithelial cells in vitro than capsule-deficient variant strains. Nonetheless, the capsules expressed by these organisms are critical to their full virulence in vivo (33).

We showed previously that antibodies to teichoic acid opsonized microencapsulated isolates of *S. aureus* for phagocytic killing in vitro (45). However, these antibodies failed to protect animals against staphylococcal endocarditis in our study and in the study reported by Greenberg et al. (21). Similarly, antibodies to teichoic acid failed to protect mice against the development of renal abscesses provoked by a microencapsulated strain of *S. aureus* (24a). Serologic studies have revealed no correlation between titers of teichoic acid antibody and immunity to staphylococcal infections in humans with invasive disease (10).

In summary, our findings indicate that antibodies to the *S. aureus* capsular polysaccharide are not protective against experimental endocarditis. Additional studies must determine whether capsular antibodies are protective in other models of staphylococcal infection. Recently, several laboratories have synthesized conjugate vaccines consisting of *S. aureus* capsular polysaccharide covalently linked to protein (13, 18, 32). Whether antibodies elicited by conjugate vaccines protect animals against staphylococcal infections remains to be determined.

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