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Role of Culture Conditions and Immunization in Experimental Nutritionally Variant Streptococcal Endocarditis

I. VAN DE RIJN

Department of Microbiology and Immunology, Wake Forest University Medical Center, Winston-Salem, North Carolina 27103

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The nutritionally variant streptococci (NVS) are usually isolated from patients with NVS endocarditis and recently have been serotyped into three groups. In the past, studies on microbial endocarditis have not analyzed the effect of the growth medium or growth phase on the bacteria used to induce the disease in the experimental rabbit model. Therefore, in this study various bacterial growth parameters were examined, including growth in semisynthetic or complex medium to the exponential or stationary phase of growth. The 50% infective dose ranged from 3.7×10^5 to 8.5×10^6 CFU for representative strains from each of the three serotypes grown under these conditions, indicating that there was no significant difference. The role of immunization was also examined in this model using organisms grown to the exponential phase in semisynthetic medium. Rabbits were immunized with heat-killed whole cells, high titers of specific antibody were produced as demonstrated by enzyme-linked immunosorbent assay, and then the rabbits were challenged with 20- to 100-fold 50% infective dose of the homologous strain. A total of 90 to 100% of the rabbits were protected from the disease process, as shown by the absence of the organisms from the heart valve 3 days after the challenge. Rabbits immunized with the amphiphile that replaces lipoteichoic acid in these organisms were not protected from challenge, demonstrating that another surface component is responsible for adherence or colonization or both. Finally NVS were incubated with radioiodinated fibronectin, fibrinogen, or laminin to determine whether these molecules aided in the adherence of the organisms to the heart valve. Only minor amounts of these components were bound to NVS as compared with controls. Therefore, NVS bind directly to the damaged heart valve or through an unknown mechanism.

The viridans group streptococci are responsible for 50 to 55% of microbial endocarditis. Among these various species, the nutritionally variant streptococci (NVS) recently have been identified as the main organisms associated with culture-negative endocarditis and are responsible for 5 to 10% of all cases of streptococcal endocarditis (18). NVS are characterized by their growth as satellite colonies around colonies of *Staphylococcus epidermidis* or several other gram-positive or gram-negative bacterial strains (12) and by the presence of a pH-dependent chromophore in their cell wall (5, 25). Recently, the NVS has been subdivided into three serotypes by rocket-line immunoelectrophoresis (RIE) and hemagglutination inhibition techniques (26). During these studies, no common group antigen was found.

The rabbit has served as the major animal model for experimental bacterial endocarditis since a reproducible technique was developed by Garrison and Freedman (13). This technique and others derived from it have served to show the role of immunization in the prevention of bacterial endocarditis for streptococci other than NVS (8, 19). Since NVS now are serotyped and since a preliminary analysis of their cell surface has been completed (24), we decided to investigate the role of bacterial growth conditions and immunization of rabbits in NVS endocarditis.

In this report, we demonstrate that the changes caused by growth of the NVS in different media and to different phases of growth have no effect on the 50% infective dose (ID_{50}) in the experimental rabbit model. In addition, animals immunized with representative heat-killed strains from each of the three serotypes were protected against NVS endocarditis. However, when rabbits were immunized with an amphiphile from the serotype I strains, no protection was elicited. Furthermore, various serum and tissue components were tested for their ability to bind to the surface of NVS. Only minor binding was demonstrated as compared with that in control strains, indicating that NVS bind directly to the heart valves or through another untested mechanism.

MATERIALS AND METHODS

Strains. The NVS strains used in this study were isolated from patients with endocarditis and have been described previously (26). Stock cultures were kept lyophilized or frozen at −80°C. For each experiment, satellite tests were performed to check the purity and stability of each strain.

The *Staphylococcus aureus* Cowan, *Streptococcus pyogenes* 1RP41, and *Escherichia coli* strains were from the culture collection of this laboratory and were stored frozen at −80°C.

Growth of bacterial strains. For preparation of inocula, vaccines, and bacteria for binding studies, NVS were grown in a semisynthetic medium (CDMT) (4) unless otherwise noted. For studies with complex medium, NVS were grown in Todd-Hewitt broth supplemented with 50 μg of pyridoxal hydrochloride per ml (THBP). Other bacteria were grown in the chemically defined medium described by van de Rijn and Kessler (27). Growth of cultures was measured with a Spectronic 20 spectrophotometer (Bausch and Lomb, Inc., Rochester, N.Y.) in 18-mm tubes at a wavelength of 650 nm.

Experimental rabbit endocarditis model. Left-sided endocarditis was induced by using modifications of the techniques described previously (7, 19). Rabbits (2.5 to 3.5 kg) were catheterized by placement of a polyethylene catheter across the aortic valve which was then permitted to remain in place for an additional 20 min. At this time the bacterial strains were injected through the catheter in a volume of 1 ml of CDMT. After an additional 10 min the catheter was

removed. The animals were sacrificed at 72 h, and the aortic valves were removed aseptically, homogenized in CDMT, and then plated on THBP. In addition, the homogenates also were incubated at 37°C for 24 h and replated. Rabbits were diagnosed positive for endocarditis if both satellite colonies were isolated from the aortic valve and the isolated organisms reacted positively in the chromophore assay.

For determination of the ID_{50} of each NVS strain, six rabbits per set were inoculated with bacteria (10^4, 10^5, 10^6, 10^7, and 10^8 CFU). The challenge dosage was preliminarily quantitated with a previously defined optical density versus viable count curve for ID_{50} and protection studies. Dilutions of the bacteria used in the experiments were made in CDMT and were subsequently spot plated (25 μl) on Todd-Hewitt broth agar plates supplemented with 5% sheep blood and 50 μg of pyridoxal hydrochloride per ml. All counts were done in quadruplicate.

The ID_{50} was calculated by the method of Probit analysis (11) while the significance of the differences of the ID_{50}s was determined at the 5% level by multiple regression analysis and the *F* test for equality of variances (3). Protection data were analyzed by the Fisher exact test (3).

Chromophore assay. Streptococci (250 ml) were grown in CDMT as described above and harvested by centrifugation at 10,000 × *g* for 5 min. The supernatant was removed, and the bacteria were suspended in a volume of 2 N HCl equal to that of the pellet. The suspension was then heated at 100°C for 5 min. The appearance of a pink to red color indicated a positive reaction.

Satellite test. NVS were characterized by the satellite test on Trypticase soy agar enriched with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.). The surface was streaked with the suspected NVS culture, overlaid with loops of *Staphylococcus epidermidis* and incubated for 18 to 48 h at 37°C. Colonies of the NVS strain only grew adjacent to the *S. epidermidis* colony. It is important only to use commercial plates or at least 2-week-old blood plates for this assay to function properly.

Preparation of inocula and immunization of rabbits. NVS strains were grown to stationary phase in CDMT (1 liter) and then sedimented at 1,500 × *g* for 10 min. The bacteria were suspended in 30 to 40 ml of phosphate-buffered saline (PBS; 0.05 M sodium phosphate, 0.15 M sodium chloride [pH 7.4]) and resedimented as described above. This process was repeated three times to remove extracellular contaminants. The bacteria were then suspended in 50 ml of PBS and heat killed at 56°C for 45 min followed by five further washes with PBS. The bacteria were finally suspended in 40 ml of PBS for immunization of rabbits.

Rabbits were immunized intravenously with the heat-killed whole cell vaccine described above three times weekly for 4 weeks. The rabbits received 0.1 ml per injection during the first week, 0.2 ml during the second week, and 0.3 ml during the third and fourth weeks. All of the above rabbits described above were bled for both preimmune and postimmune sera for the antibody studies.

For immunization of the rabbits with the amphiphile, NVS-47 (the prototype strain for serotype I) served as the source of the amphiphile. NVS-47 was grown at 37°C to late stationary phase in a semisynthetic medium (CDMT, 60 liters) and was separated from the extracellular fluid with a Sharples high speed centrifuge (Sharples-Stokes Div., Pennwalt Corp., Warminster, Pa.) at 4°C. The extracellular products were also collected and served as the source of the amphiphile.

Next, the extracellular fluid containing the amphiphile was concentrated with an Amicon DC 10L concentrator equipped with a H10P30-20 hollow fiber cartridge (30,000-molecular-weight cutoff). Once the material was concentrated to 500 ml it was dialyzed extensively against distilled water and filtered through a 0.22-μm membrane to remove any residual bacteria, and then sodium acetate was added to a final concentration of 1%. The amphiphile was concentrated further by precipitation with three volumes of ice cold acetone, the resulting precipitate was collected by centrifugation at 6,000 × *g*, and the residual acetone was removed by desiccation in vacuo. This preparation served as the source of the NVS serotype I amphiphile for all the experiments.

Rabbits were immunized with amphiphile intravenously (1 ml) for 4 weeks at weekly intervals through the marginal ear vein by the double adjuvant technique described by Fiedel and Jackson (10).

Analysis of rabbit sera for antibodies against the immunogen. The antisera were analyzed for antibody by RIE as described previously (21). Furthermore, the antisera were quantitated for bacterial surface antibody by an enzyme-linked immunosorbent assay (ELISA). The ELISA assay for detection of antibodies against NVS and the serotype I amphiphile was accomplished by a modification of the procedure described by Voller et al. (29) as follows. Bacteria (optical density at 650 nm, 0.5; 1:10 dilution) or amphiphile (5 μg/ml) were coated onto 96-well microtiter plates with a 0.05 M sodium carbonate buffer (pH 9) containing 0.02% sodium azide. The antigen was incubated at 4°C for 18 h followed by three washes with 0.05 M sodium phosphate–0.15 M sodium chloride–0.05% Tween 20 (pH 7.4) buffer (PBST). Any exposed sites on the plates were then covered with a solution of 5% bovine serum albumin (BSA; ELISA grade, Sigma Chemical Co., St. Louis, Mo.) in PBST at 37°C for 1 h. Excess BSA was removed by three further washes with PBST followed by incubation of the diluted antisera for 3 h at 37°C. All dilutions were made in 1% BSA-PBST. The plates were then washed four times with PBST; and a mixture of peroxidase-conjugated specific antibody to immunoglobulin G (Bionetics) was diluted 1:750 in 1% BSA-PBST, added to the plates, and incubated for an additional 3 h at 37°C. Finally the plates were washed four times with PBST and one time with PBS, and then they were incubated with *ortho*-dianisidine dihydrochloride for 15 min at 37°C for color development. The plates were read on a Titertek Multiskan MC with a 450-nm filter. The reciprocal of the last dilution with a reading of 0.1 was considered the titer of the serum.

Sensitization of sheep erythrocytes. Sheep erythrocytes were collected in Alsever's, washed four times with PBS, and diluted to a 50% suspension of the erythrocytes. A 0.4-ml fraction of the suspended erythrocytes was mixed with the purified amphiphile (200 μl, 2 mg/ml) in PBS to give a final 2% (vol/vol) suspension of erythrocytes. Controls containing no added extract were also prepared, and the mixtures were rotated at 37°C for 1 h followed by standing at 4°C overnight. The erythrocytes were then washed four times with 0.85% NaCl and finally suspended to 1% (vol/vol) in PBS.

Hemagglutination assays. Duplicate serial dilutions of serum were prepared in PBS in microtiter plates. To each dilution of antisera was added an equal volume of either sensitized or control erythrocytes. The reactants were mixed and incubated for 1 h at 37°C. The reciprocal of the greatest dilution showing agglutination on macroscopic inspection was recorded as the endpoint or titer.

Isolation and radioiodination of serum and tissue components. Human fibronectin was isolated from plasma by gelatin-agarose chromatography by the technique of Engvall and Ruoslahti (9). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis determined that the product was pure. Human fibrinogen was provided by R. Hantgan (Wake Forest University Medical Center), while laminin was provided by L. Switalski (University of Alabama, Birmingham, Ala.).

Each of these components was radiolabeled with ^{125}I using the lactoperoxidase-glucose oxidase radioiodination reagent (Bio-Rad Laboratories, Richmond, Calif.). Free radiolabel was removed from bound label by Sephadex G50 chromatography on a column precoated with BSA. The radiolabeled component was isolated, and the counts per minute per nanogram were determined.

Binding assay. Radioiodinated serum and tissue components were incubated with bacteria (150 μl, 10^9 CFU/ml) for 60 min at 37°C in the presence of 0.05% BSA–0.05 M sodium phosphate (pH 7.4). Free serum or tissue components were then separated from their bound counterparts by filtration with a Millipore millititer plate (0.22-μm membrane, 96-well; Millipore Corp., Bedford, Mass.). Six volumes of PBS containing 0.05% BSA were used to remove any remaining unbound components. The individual membranes were then excised from the plate and counted for radioactivity. All assays were performed in triplicate.

RESULTS

Effect of bacterial culture conditions on induction of endocarditis. A representative strain from each of the three NVS serotypes was grown in either CDMT or THBP to the exponential or stationary phase of growth. The bacteria were then diluted in fresh medium to the appropriate concentration (10^4, 10^5, 10^6, 10^7, and 10^8 CFU/ml), and sets of six rabbits each were challenged with 1 ml of the bacterial dilution. At 3 days postchallenge the rabbits were sacrificed, and their heart valves were analyzed for the presence of vegetations containing the NVS. ID_{50} values were calculated by the technique of Probit analysis (11) and are shown in Table 1.

The ID_{50} of the serotype I strain varied from 4.5×10^6 to 7.4×10^6 CFU in CDMT and from 1.4×10^6 to 3.7×10^5 CFU in THBP when grown to the exponential and stationary phases, respectively. The ID_{50} of the serotype II strain varied from 8.5×10^6 to 3.9×10^6 CFU in CDMT and 1.3×10^6 to 2.8×10^6 CFU in THBP at the two growth phases, respectively. These data and those for the serotype III strain did not show any significant differences at the 5% level when the ID_{50}s were compared between media or growth phases. In addition no consistent trends were observed other than the possibility that slightly more bacteria grown in CDMT were required to cause endocarditis than the number of bacteria grown in THBP. During trial runs there was no variation in the observed ID_{50} whether the organisms were diluted in medium or saline (data not shown).

TABLE 1. ID_{50} of NVS strains grown in CDMT or THBP and harvested during the exponential or stationary phase of growth

Serotype	Strain	Medium	Phase of growth	ID_{50} (CFU)
I	NVS-47	CDMT	Exponential	4.5×10^6
			Stationary	7.4×10^6
		THBP	Exponential	1.4×10^6
			Stationary	3.7×10^5
II	NVS-63	CDMT	Exponential	8.5×10^6
			Stationary	3.9×10^6
		THBP	Exponential	1.3×10^6
			Stationary	2.8×10^6
III	NVS-69	CDMT	Exponential	4.1×10^6
			Stationary	4.9×10^6

[a] The ID_{50} was calculated by using six rabbits at each dilution of bacteria (10^4, 10^5, 10^6, 10^7, and 10^8). A rabbit was considered positive for endocarditis if the challenge organism was isolated from the heart valve 3 days after inoculation.

TABLE 2. ELISA titer of preformed antibody to NVS and the serotype I amphiphile

Immunogen	Mean titer (± SEM[a])
Serotype I	
NVS-47 heat-killed cells	532,480 ± 59,357
Preimmune	<200
NVS-47 amphiphile	10,640 ± 2,842
Preimmune	5 ± 2.5
Serotype II	
NVS-63 heat-killed cells	286,720 ± 40,445
Preimmune	<200
Serotype III	
NVS-69 heat-killed cells	281,600 ± 35,054
Preimmune	<200

[a] SEM, Standard error of the mean.

Immunization of rabbits. Rabbits were immunized intravenously with either heat-killed whole cell vaccines or preparations of partially purified NVS serotype I amphiphile. After a 4-week immunization schedule, the rabbit sera were analyzed for specific antibody by both RIE and ELISA. The rabbits immunized with the amphiphile showed a single precipitin peak by RIE, whereas the rabbits immunized with the whole cell vaccines exhibited multiple peaks by this technique.

Quantitation of specific antibody in the respective antisera by ELISA demonstrated that each of the rabbits contained high titers of antibodies after immunization as compared with those during their preimmune state (Table 2). The mean titers of antibody against whole cells of NVS were 532,480, 286,720, and 281,600 for serotypes I, II, and III, respectively. None of the preimmune sera contained any demonstrable anti-NVS antibody at a dilution of 1:200.

Sera from rabbits immunized with the partially purified serotype I amphiphile were assayed for antibody against their immunogen. These sera contained a mean antibody titer of 10,640 as compared with a mean titer of 5 for the preimmune sera. Furthermore, hemagglutination titers of 5,000 to 10,000 were demonstrated for these antisera (data not shown).

Since sera from the rabbits were demonstrated to contain elevated antibodies titers against the respective immunogen, these rabbits were used to analyze the protective effect of the immunization protocols.

Protection against endocarditis in rabbits. The rabbits described above were challenged with a dose of bacteria equivalent to that required to induce 100% endocarditis (20 to 100 times the ID_{50}). In each case the challenge organism was the same as the immunizing strain. Three days later the rabbits were sacrificed, the hearts were removed, and the heart valves were analyzed for vegetations and the challenge strain.

TABLE 3. Protection of rabbits against NVS endocarditis by immunization with heat-killed whole cells or an amphiphile preparation[a]

Immunogen	Protection	
	No. protected/ no. challenged (percent)	Significance
Serotype I		
NVS-47 heat-killed cells	9/10 (90)	$P < 0.001$
NVS-47 amphiphile	1/8 (13)	NS[b]
NVS-47 unimmunized control	0/10 (0)	
Serotype II		
NVS-63 heat-killed cells	10/10 (100)	$P < 0.001$
NVS-63 unimmunized control	1/10 (10)	
Serotype III		
NVS-69 heat-killed cells	8/8 (100)	$P < 0.001$
NVS-69 unimmunized control	0/10 (0)	

[a] Rabbits were analyzed for the presence and titers of antibody of various cell surface components before they were challenged with a 20 to 100-fold larger dose than the ID_{50} of exponential-phase (CDMT) cells. Rabbits were considered positive for endocarditis if the challenge organism was isolated from the heart valve 3 days after inoculation.
[b] NS, Not significant.

Of 10 rabbits immunized with the serotype I strain, 9 (90%) were protected when challenged with live organisms (Table 3). In contrast, all 10 nonimmunized rabbits developed endocarditis. Since lipoteichoic acid has been demonstrated to be involved in the adherence of certain streptococci to epithelial cells (6, 16), rabbits were immunized with the NVS serotype I amphiphile to determine whether this surface component is involved in the adherence process. Once high titers of specific antibody were produced, the rabbits were challenged with serotype I bacteria. Of these rabbits, only one of eight was protected, indicating that with this strain the amphiphile is not involved in adherence of the bacteria to the damaged heart valve.

The studies described above were repeated with representative serotype II and III strains. In both cases 100% of the rabbits with preformed specific antibody were protected (serotype II, 8 of 8; serotype III, 10 of 10). Of 10 unimmunized rabbits, 1 did not develop endocarditis when challenged with the serotype II organism, while all 10 control rabbits challenged with the serotype III organism developed endocarditis.

Statistical analysis of the results indicate that all of the rabbits immunized with heat-killed whole cells were significantly protected as compared with their unimmunized controls ($P < 0.001$). There was no significant protection demonstrated with the amphiphile-immunized rabbits.

Ability of the NVS to bind serum and tissue components. In the past, it has been suggested that bacteria bind to heart valves because of their affinity for certain serum and tissue components (28). To analyze whether this plays a role in the adherence of NVS to the damaged heart valve, fibronectin, fibrinogen, and laminin were radioiodinated and incubated with the NVS strains; and the amount of each component bound to the NVS strains was quantitated.

The data obtained from these studies (Table 4) indicate that the NVS strains only bound low levels, if any, of the three serum and tissue components to their surface. The positive control stains *Staphylococcus aureus* and *Streptococcus pyogenes* bound 187 ng of fibronectin each; 233 and 840 ng of fibrinogen, respectively; and 191 ng of laminin for the *Streptococcus pyogenes* strain. The *E. coli* strain bound only low levels of the three components. These data indicate that NVS bind directly to the endothelial cells of the heart valve or through an untested serum protein.

DISCUSSION

The rabbit endocarditis model devised by Durack et al. (7), as modified by Scheld et al. (19), has been shown to be applicable for studying protection against NVS endocarditis through immunization. A total of 90 to 100% of the animals preimmunized with heat-killed whole cells of NVS were protected from NVS endocarditis when challenged with 20- to 100-fold ID_{50} of the homologous organism. At this challenge dose essentially all of the unimmunized control rabbits (29 of 30) had endocarditis 3 days after challenge. These results were significant at a level of $P < 0.001$ by the Fisher exact test.

The results from this study were well resolved as compared with those of similar studies in which other organisms were used. Initially it was believed that the presence of antibody enhanced the susceptibility of the individual to subacute endocarditis. Wadsworth has noted that when horses are immunized with pneumococci they have a greatly increased frequency of pneumococcal endocarditis (30). These studies have been corroborated by the work of Mair with rabbits (15). Keefer (14) and more recently Weinstein and Schlesinger (31) have hypothesized from these and results of other studies that the presence of antibody in the blood enhances the incidence of endocarditis because of the ability of antibodies to agglutinate the organisms. This aggregate could then associate with a scarred or congenitally deformed heart valve.

More recently, results of experiments by Durack et al. (8) with *Streptococcus sanguis* and *Streptococcus mutans*, Scheld et al. (19) with *Streptococcus sanguis*, and Adler et al. (1) with pneumococci have demonstrated that rabbits preimmunized with these organisms are protected to various degrees against the challenge dose. Other studies by Thorig et al. (23) with *Streptococcus sanguis* and *Staphylococcus*

TABLE 4. Human ^{125}I-labeled serum and tissue components binding to NVS and control strains

Strain	Binding (ng) of the following components[a]:		
	Fibronectin	Fibrinogen	Laminin
Serotype I			
NVS-47	14.9	2.4	1.0
NVS-52	4.6	15.2	0.2
Serotype II			
NVS-63	0	15.4	1.4
NVS-70	16.4	11.4	0.7
NVS-71	0	55.5	2.0
Serotype III			
NVS-69	7.6	0	2.5
Staphylococcus aureus	186.8	233.4	ND[b]
Streptococcus pyogenes	187.5	840.0	191.3
Escherichia coli	0	24.2	1.6

[a] Amount bound per 1.5×10^8 CFU.
[b] ND, Not done.

epidermidis and Thorig et al. (22) with *E. coli* have demonstrated that preimmunization has no effect on the protection of the rabbits against endocarditis. Thorig et al. (23) have suggested that the inconsistency between the sets of experiments with *Streptococcus sanguis* is due to differences in the procedure to induce and infect the vegetations. This variation in the results as yet has not been resolved. Finally, Archer and Johnston (2) have demonstrated that preimmunization of rabbits with *Pseudomonas aeruginosa* prolonged the disease process instead of protecting the rabbits. Therefore the role of immunization in a number of other microbial endocarditis models is not yet clear.

One of the major aims of these studies was to determine whether the growth medium or growth phase of the bacteria had an effect on the ID_{50} for NVS endocarditis. Experiments with representative strains from the three serotypes of NVS demonstrated that there were no significant differences between the ID_{50} of organisms grown in CDMT versus those grown in THBP or between exponential and stationary phases. Wet mount preparations of the challenge dose showed that the average chain length was independent of the growth conditions for this set of experiments and that this factor need not be considered in evaluating the results.

Recent quantitative analyses of the cell walls of NVS have demonstrated that, in addition to the usual streptococcal peptidoglycan components, glucosamine, glucose, galactose, ribitol, phosphorus, and a small amount of rhamnose were also present (24). Galactosamine was found only in serotype II and III cell walls. These studies demonstrate that qualitative changes do not occur in the cell walls of NVS grown in these two media to the various phases of growth, even though quantitative differences do occur. Therefore, the surface components involved in the pathogenesis of endocarditis are stable to the point that any variation does not affect the ID_{50}.

Capsules have been implicated in the adherence process of other streptococci to heart valves (17, 19). Since the NVS do not appear to produce a capsule, as determined by Indian ink staining and the absence of a Quellung reaction, other surface component(s) are responsible for the attachment of the bacteria to the heart valve. In an initial attempt to identify the NVS surface component(s) responsible for eliciting the protective antibody, the NVS serotype I amphiphile was isolated and purified. This unique amphiphile replaces lipoteichoic acid in these organisms and was chosen for these studies because one of the roles that lipoteichoic acid plays in streptococci is as a mediator in the adherence process (6, 16). Rabbits were immunized with the amphiphile, and once high titers of antibody were present, the rabbits were challenged with serotype I organisms. The amphiphile afforded the rabbits no protection, indicating that it probably does not play a role in the adherence of the organism to the heart valve. Currently, other surface components are being isolated to test their possible role as protective component(s).

It has been suggested by Vercellotti et al. (28) that the viridans group streptococci bind to cardiac valves via fibronectin. Results of this study indicate that the NVS strains lack receptors for various serum and tissue components, including fibronectin. Whether these strains have the ability to bind directly to endothelial cells or constituents of scar tissue remains an area for future experimentation. We examined the adherence of the NVS in the fibrin-platelet adherence assay as described by Scheld et al. (20). Unfortunately, the NVS bound at low levels in this assay, and the results were variable and not reproducible (data not shown).

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