Resistance of *Streptococcus gordonii* to Polymorphonuclear Leukocyte Killing Is a Potential Virulence Determinant of Infective Endocarditis

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Significant differences in virulence among seven representative *Streptococcus gordonii* strains were observed by using the rat model of infective endocarditis. Five strains, including *S. gordonii* DL1, caused severe disease, while the other two strains, including *S. gordonii* SK12, caused minimal or no disease. The differences in virulence were evident from the visible presence of streptococci in the vegetations present on the aortic valves of catheterized rats that were challenged with individual strains and also from the much greater recovery of rifampin-resistant *S. gordonii* DL1 than of streptomycin-resistant *S. gordonii* SK12 from the hearts of animals coinfected with both organisms. Each *S. gordonii* strain aggregated with human platelets and bound to polymorphonuclear leukocytes (PMNs), as shown by the stimulation of PMN superoxide anion production. These interactions were reduced or abolished by pretreatment of the platelets or PMNs with sialidase, indicating that there was bacterial recognition of host sialic acid-containing receptors. Adhesin-mediated binding of each *S. gordonii* strain to PMNs also triggered phagocytosis. However, the subsequent PMN-dependent killing differed significantly for the seven strains. The five virulent strains included three strains that were not killed and two strains whose numbers were reduced by approximately 50%. In contrast, the level of killing of each avirulent strain under the same conditions was significantly greater and approached 90% of the bacteria added. Parallel studies performed with rat PMNs revealed comparable differences in the resistance or susceptibility of representative virulent and avirulent strains. Thus, the ability of *S. gordonii* to survive in PMNs following adhesin-mediated phagocytosis may be an important virulence determinant of infective endocarditis.

The presence of endocardial vegetations composed of a fibrin-platelet matrix, leukocytes, and bacteria is the hallmark of infective endocarditis (13, 15). The predisposing factors for the initiation of infection include several conditions that damage the endocardial surface, including left ventricular catheterization in animal models, which induces thrombus formation on the aortic valve (32). Insight into the microbial colonization of such sites was gained from histological examination of heart valves fixed 30 min after intravenous challenge of catheterized rabbits with a viridans group streptococcus (6). Interestingly, most of the streptococci observed at this time were not attached to endocardial thrombi but instead were present within associated polymorphonuclear leukocytes (PMNs) or monocytes. In contrast, similar vegetations on heart valves fixed 1 day after challenge contained dense masses of infecting streptococci in localized regions of agranulocytosis. Thus, the ability of these bacteria to resist or avoid host cellular and humoral defenses may be an important virulence determinant of infective endocarditis.

*Streptococcus sanguinis* and *S. gordonii* are among the most common streptococcal species currently recognized as etiological agents of infective endocarditis (5, 31). Interactions between these bacteria and platelets represent a potentially important step in pathogenesis (8, 9). Recently, such interactions have been found to involve the binding of streptococcal adhesins identified as large serine-rich glycoproteins (2, 19, 25) to platelet membrane sialoglycoproteins (1, 19, 29, 34). Moreover, deletion of the gene (*hsa*) for the sialic acid-binding adhesin of *S. gordonii* DL1 has been shown to significantly reduce the infectivity of this strain in the catheterized rat model of infective endocarditis (28). In addition to platelet membrane components, the *S. gordonii* DL1 adhesin recognizes other surface-associated sialoglycoproteins (10, 26, 27), including the α2,3-linked sialic acid termini of leukosialin and leukocyte common antigen on human PMNs (20). While the biological consequences of this interaction are not known, adhesin-mediated binding of another oral species, *Actinomyces naeslundii*, to galactose termini of the same PMN surface components triggered activation of these host cells (22), resulting in phagocytosis and killing of this microorganism (21).

In this study we found significant differences in the virulence of representative *S. gordonii* strains in the rat model of infective endocarditis. Importantly, these differences do not appear to be correlated with the adhesion of these bacteria to isolated platelets or the fibrin-platelet matrix but instead are correlated with the biological consequence of bacterial binding to PMNs. The findings suggest that the ability of *S. gordonii* to survive in PMNs following adhesin-mediated
phagocytosis may be an important virulence determinant of infective endocarditis.

**MATERIALS AND METHODS**

**Bacteria.** The seven wild-type strains of *S. gordonii* used in this study have all been described previously (4, 10, 11). To our knowledge, the origin of *S. gordonii* DL (Challis) is not known. The other strains are oral isolates. Spontaneous rifampin-resistant mutants of *S. gordonii* DL, SK3, and SK9 and streptomycin-resistant mutants of *S. gordonii* 38, K4, SK186, and SK12 were selected on brain heart infusion (BHI) agar containing 50 μg/mL rifampin or 100 μg/mL streptomycin. The adhesive properties of mutant and corresponding parental strains were indistinguishable in various in vitro assays, including assays for coaggregation with other oral bacteria, bacterium-mediated hemagglutination of human erythrocytes, and bacterium-mediated aggregation of proline-rich protein-coated latex beads (10). The levels of growth of parent and mutant strains in antibiotic-free complex medium containing tryptone, yeast extract, Tween 80, and glucose (0.2%) buffered to pH 7.5 with K_2HPO_4 (4) were also the same based on generation times calculated from measurements of culture turbidity obtained with a Klett-Summon colorimeter.

**Catheterized rat model of infective endocarditis.** Male Sprague-Dawley rats (350 to 400 g) were anesthetized with methoxyflurane (Methofane; Pitman-Moore, Mundelein, IL) and sodium pentobarbital (Somnifer; Richmond Veterinary Supply, Richmond, VA) prior to left ventricular catheterization, which was performed as previously described (7, 14, 23, 35) to damage aortic valves. Catheters consisting of Intramedic PE10 polyethylene tubing (Clay Adams, Parsippany, NJ) were passed through the right common carotid artery into the left ventricle and advanced toward the aortic root. One day later, catheterized animals were challenged by injection of individual *S. gordonii* strains (1 mL containing from 3 × 10^8 to 1 × 10^9 CFU) in the femoral vein. Individual animals were also challenged intravenously with 1 mL containing approximately 1 × 10^8 CFU of rifampin-resistant *S. gordonii* DL1 and 1 × 10^9 CFU of streptomycin-resistant *S. gordonii* SK12. The streptococci used in these studies were harvested by centrifugation of overnight broth cultures (4), washed with RPMI 1640 (Whittaker Bioproducts, Walkersville, MD), sonicated briefly to disrupt cell walls, and harvested by centrifugation of overnight broth cultures (4), washed with RPMI 1640 containing 0.05% Tween 20, and plated on BHI agar containing 50 μg/mL kanamycin and 3 μg/mL cefoxitin to allow the maintenance of antibiotic resistance markers. The overall type 1 error rate. The levels of recovery of one-way analysis of variance model. All comparisons between pairs of strains were independently at least four times. PMN-dependent killing of each strain was calculated from differences in the decrease of bacterial cell counts. For each experiment and to confirm the maintenance of antibiotic resistance markers.

**Animals.** Animals were sacrificed 48 h after challenge and examined to determine whether the catheters were positioned properly. The only animals included in the present study were animals in which the tip of the catheter was positioned in the middle of the left ventricle. Organs (heart, spleen, and liver) were harvested, weighed, transferred to small tubes of RPMI 1640, homogenized at 9,500 rpm with a Tissumizer Mark II (Tekmar Co., Cincinnati, OH), sonicated three times for 10 s using a Micro-Ultrasonic cell disruptor (Kontes, Vineland, NJ), diluted with RPMI 1640 containing 0.05% Tween 20, and plated on BHI agar containing 50 μg/mL rifampin or 100 μg/mL streptomycin. Individual organs were collected from the jugular vein in small heparin-containing tubes, sonicated three times for 10 s, and plated as described above. CFU were counted following incubation of the plates for 3 days at 37°C. The amounts of individual strains recovered from the hearts of catheterized rats, expressed as log_{10} CFU/g of heart, were compared using a one-way analysis of variance model. All comparisons between pairs of strains were performed using the Tukey multiple-comparison procedure to control for the overall type 1 error rate. The levels of recovery of *S. gordonii* DL1 and *S. gordonii* SK12 from individual animals were also compared; for between-group comparisons of the uncatheterized and catheterized groups two-sample t tests were used, and for within-group comparisons paired t tests were used. Tissues were also fixed in 10% neutral buffered formalin and embedded in paraffin, and 4-μm sections were examined by light microscopy.

**Bacterial clearance.** Each of 20 uncatheterized rats was inoculated as described above with approximately 1 × 10^8 CFU of rifampin-resistant *S. gordonii* DL1 and 1 × 10^6 CFU of streptomycin-resistant *S. gordonii* SK12. Blood, spleen, and liver samples from four rats at each of five times (10 min, 30 min, 2 h, 6 h, and 24 h postinjection) were prepared as described above and plated on rifampin- and streptomycin-containing BHI agar to compare the clearance of strain DL1 with the clearance of strain SK12.

**Platelet aggregation.** The platelet bacterial adhesion assay (29) was performed with either freshly isolated or outdated platelets (Department of Transfusion Medicine, National Institutes of Health, Bethesda, MD), both of which gave the same results. The platelets were either not treated or treated with 0.5 U/ml sialidase (type X neuraminidase from Clostridium perfringens; Sigma-Aldrich) for 2 h at room temperature to remove sialic acid. Reaction mixtures (100 μL) containing platelets (1 × 10^7 cells) and bacteria (1 × 10^7 CFU) were set up in individual wells of flat-bottom, 96-well plates, incubated for 2 h at room temperature with periodic mixing on a shaker to promote aggregation, and photographed.

**Adhesion of bacteria to fibrin-platelet matrix.** A fibrin-platelet matrix was prepared using previously described protocols (3, 24, 30) by mixing 1.0 mL of fibrinogen with thrombin [Sigma-Aldrich, St. Louis, MO] in saline containing 0.125 M CaCl_2 in individual 60-mm petri dishes. The dishes were incubated for a few minutes at room temperature and then for 30 min at 37°C to allow clot formation, and they were stored overnight at 4°C. Dishes containing a fibrin-platelet matrix were rinsed three times with 5 mL Hank’s balanced salt solution (HBSS) (BioWhitaker, Inc., Walkersville, MD) containing 1 mM CaCl_2, 1 mM MgCl_2, and 0.5% bovine serum albumin (BSA), incubated for 45 min at 37°C with approximately 1 × 10^6 CFU of each *S. gordonii* strain, Staphylococcus aureus Cowan 1, or Escherichia coli ATCC 25922 (30) in 5 mL of the same buffer, washed three times with buffer to remove unbound bacteria, and overlaid with 6 mL of warm BHI agar. Colonies resulting from the growth of bound bacteria were counted after incubation of the plates for 1 day (E. coli) or 2 days (streptococci and staphylococci) at 37°C. Values for bound bacteria, expressed in CFU/plate, were pooled from four independent experiments and compared using a two-way analysis of variance model, which allowed us to control for differences among experiments. Pairwise comparisons among the nine strains examined were conducted using the Tukey multiple-comparison procedure.

**Superoxide anion production by PMNs.** PMNs were isolated by Ficol-His-topaque 1083 (Sigma-Aldrich) separation of human buffy coats (Department of Transfusion Medicine, National Institutes of Health, Bethesda, MD), followed by lysis of erythrocytes with NH_4Cl lysing buffer (12). Stimulation of PMN superoxide anion production was determined as previously described (22) using quadruplicate sets of reaction mixtures (1.5 mL) that contained PMNs (approximately 6 × 10^6 cells) and bacteria (approximately 2 × 10^9 CFU) in HBSS supplemented with 0.15 mM CaCl_2, 1 mM MgCl_2, and 1% BSA. Reaction mixtures with and without bacteria were pretreated for 2 h at 37°C with a rotator to allow superoxide anion production. Ferricytochrome c (Sigma-Aldrich) was then added to one duplicate set of reaction mixtures, and ferricytochrome c in the presence of superoxide dismutase (Sigma-Aldrich) was added to the second duplicate identical set. The concentrations of released superoxide anion were calculated from differences in the reduction of ferricytochrome c in the presence and in the absence of superoxide dismutase as determined from measurements of the optical density at 549 nm (16). Values for superoxide production by PMNs in the presence of bacteria were corrected for the small amount of superoxide produced by PMNs in the absence of bacteria, which was always less than 0.1 μM. The bacteria in some reaction mixtures were pretreated with 1.0 mg/mL of pronase (Calbiochem, San Diego, CA) for 2 h at 37°C, washed three times, and suspended in HBSS prior to incubation with PMNs. The PMNs in other reaction mixtures were pretreated with 1% (v/v) trypsin (Sigma, St. Louis) for 30 min at 37°C, washed three times, spun down, and suspended in HBSS containing 0.15 mM CaCl_2, 1 mM MgCl_2, and 1% BSA prior to incubation with bacteria. Sialylactose (Boehringer, Mannheim, Germany) and other saccharides were tested as potential inhibitors at a final concentration of 5.0 mM in other reaction mixtures. Levels of PMN superoxide anion production in experimental reaction mixtures containing polymyxin-treated bacteria, sialidase-treated PMNs, or individual saccharides and control reaction mixtures containing untreated PMNs and bacteria were compared by two-sample t tests. A Bonferroni correction (α = 0.05/21) was used to control the type 1 error rate for the 21 comparisons.

**Bactericidal assay.** Reaction mixtures (1.5 mL) containing bacteria (approximately 2 × 10^8 CFU) and untreated or sialidase-treated PMNs (approximately 6 × 10^6 cells) and control mixtures containing bacteria alone were set up in 3-mL tubes containing 1.5 mL RPMI 1640 supplemented with 9 mM HEPES (Whittaker Bioproducts) and 1% BSA. The tubes were incubated on a rotator for 2 h at 37°C to allow phagocytosis. The mixtures were sonicated three times for 10 s to disrupt the PMNs, diluted in RPMI 1640 containing 0.05% Tween 20, and plated on BHI agar using a spiral plater (Spiral Systems, Cincinnati, OH) (12, 21). Colonies were counted after incubation of the plates for 3 days at 37°C. Bactericidal assays were performed in duplicate and were repeated independently at least four times. PMN-dependent killing of each *S. gordonii* strain was calculated by performing a two-way analysis of variance of viable bacteria (i.e., log_{10} CFU) in reaction mixtures containing bacteria alone or bacteria plus PMNs. To test whether *S. gordonii* SK9 and SK12 were more susceptible to killing than *S. gordonii* K4 and SK33, a test was performed on the linear function comparing the two groups of means.
Rat PMNs were isolated from heparinized blood by the methods used for human PMNs. The rat cells obtained consisted of more than 97% PMNs, as shown by staining with a Diff-Quik stain set (Baxter Healthcare Corp., Miami, FL). The viability of each of these preparations was more than 95%, as determined by trypan blue exclusion. Bactericidal assays with rat PMNs were performed and evaluated by using the procedures described above, except that the reaction mixtures were incubated at 37°C for 3 h instead of 2 h.

Electron microscopy. Reaction mixtures (1.5 ml) containing PMNs (approximately 1.2 × 10^7) and bacteria (approximately 3 × 10^7 CFU) were incubated on a rotator for 30 min at 37°C to allow phagocytosis and then centrifuged at low speed for 10 min. Cell pellets were treated with two changes of Karnovsky's fixative (4% paraformaldehyde, 2% glutaraldehyde in 0.1 M cacodylate buffer; pH 7.3) for 30 min, suspended in 0.1 M cacodylate buffer (pH 7.3), dehydrated in a graded alcohol series, embedded in epoxy resin, and sectioned prior to electron microscopic examination (21).

RESULTS

Virulence of S. gordonii strains in the rat model of infective endocarditis. We compared the infectivities of seven S. gordonii strains in the rat model of infective endocarditis by challenging groups consisting of four catheterized and four uncatheterized rats with individual antibiotic-resistant variants of these bacteria. The resulting infections were assessed 2 days later by plating heart, blood, liver, and spleen samples on antibiotic-containing selective media. Differences in virulence among the S. gordonii strains were not evident from the infections observed in uncatheterized animals following intravenous challenge with from 3 × 10^5 to 1 × 10^7 CFU of individual strains (Fig. 1A). Bacteria were not isolated from the blood of these animals, but relatively low numbers (<10^4 CFU per g) were isolated from heart, liver, or spleen samples. In contrast, the numbers of bacteria recovered from catheterized animals were much greater for S. gordonii 38, DL1, K4, SK133, or SK186 (Fig. 1B) than for either S. gordonii SK9 or SK12 (Fig. 1B). This was most apparent from the numbers of bacteria in the hearts of catheterized animals, which varied from 10^9 to 10^10 CFU/g for the five virulent strains but were 10^6 CFU/g or less for strains SK9 and SK12. The difference between the two groups of strains was highly significant (P < 0.05, as determined by the Tukey method, for all pairwise comparisons between strain SK9 or SK12 and each of the other five strains).

Histological examination of rat hearts revealed the presence of sterile endocardial vegetations on the aortic valves of animals that were catheterized but not challenged with bacteria (Fig. 2A). The vegetations present in comparable animals challenged with S. gordonii DL1 were much larger and contained dense masses of infecting streptococci (Fig. 2B). In contrast, streptococci were not seen in the vegetations from animals infected with S. gordonii SK12 (Fig. 2C).

To compare the levels of virulence of different strains under identical conditions, we challenged individual uncatheterized and catheterized rats with similar numbers of rifampin-resistant S. gordonii DL1 and streptomycin-resistant S. gordonii SK12 CFU. Two days later, much greater numbers of rifampin-resistant CFU than of streptomycin-resistant CFU were isolated from each animal in the catheterized group (Fig. 3). On average, the recovery of these strains from the hearts of five catheterized animals was approximately 50,000-fold greater for S. gordonii DL1 than for S. gordonii SK12, and the associated difference for four uncatheterized animals was only 10-fold. The results of further studies showed that both S. gordonii strains were rapidly cleared from the circulation of uncatheterized animals and accumulated in the liver and spleen (Fig. 4). The numbers of rifampin-resistant and streptomycin-resistant CFU recovered from these sites declined from 6 h to 24 h, and there was no obvious difference between the two strains.

Interactions of S. gordonii strains with platelets and fibrinogen-coated platelet matrix. In further comparative studies of the seven S. gordonii strains to identify in vitro correlates of virulence, we focused on possible differences in the adhesive properties of
these bacteria. In previous studies (29), the aggregation of \textit{S. gordonii} DL1 with human platelets was shown to depend on the sialic acid-binding adhesin of this strain. In the present study, \textit{S. gordonii} DL1 and each of the other \textit{S. gordonii} strains aggregated with untreated platelets but not with sialidase-treated platelets (Fig. 5, inset), confirming the involvement of sialic acid-binding adhesins in platelet binding. In other experiments, sterile clots that formed in vitro in small culture dishes were incubated with equivalent numbers of individual strains for 45 min, rinsed to remove unbound bacteria, overlaid with warm BHI agar, and incubated at 37°C to allow the attached organisms to grow. The numbers of attached cells of \textit{S. gordonii} strains were similar, and the average was around 185 CFU per plate (Fig. 5). This value was significantly less than the 350 CFU/plate observed with adherent \textit{S. aureus} Cowan 1 but more than the 17 CFU/plate observed with nonadherent \textit{E. coli} 25922 ($P < 0.05$, as determined by the Tukey method). Thus, the differences in virulence among the \textit{S. gordonii} strains were not correlated with the binding of these bacteria to isolated platelets or the fibrin-platelet matrix.

![Figure 2](image1.png)

**FIG. 2.** Hematoxylin- and eosin-stained sections of hearts from catheterized rats that were fixed 48 h after challenge with saline (A), \textit{S. gordonii} DL1 (B), or \textit{S. gordonii} SK12 (C). Sections were photographed at magnifications of $\times 50$ (left panels) and $\times 630$ (right panels). The aorta (a), aortic valve (v), and thrombus (t) that resulted from left ventricular catheterization of each animal are identified. Streptococci (s) were seen only in endocardial thrombi of rats infected with \textit{S. gordonii} DL1 (B).

![Figure 3](image2.png)

**FIG. 3.** Comparative infectivities of \textit{S. gordonii} DL1 and \textit{S. gordonii} SK12 in four uncatheterized (control) and five catheterized rats. Animals were sacrificed 48 h after challenge with a mixture of $1.1 \times 10^8$ CFU of rifampin-resistant \textit{S. gordonii} DL1 and $0.8 \times 10^6$ CFU of streptomycin-resistant \textit{S. gordonii} SK12. Mean numbers of CFU/g of heart tissue and standard deviations (error bars) were determined by duplicate plate counting on rifampin- and streptomycin-containing BHI agar.
Adhesin-mediated interactions of *S. gordonii* strains with PMNs. The sialic acid-binding adhesin of *S. gordonii* DL1 has also been shown to mediate the binding of this strain to human PMNs (20). The present findings confirmed and extended this finding by showing that the interaction of this *S. gordonii* strain and each of the other strains with human PMNs stimulated the production of superoxide anion (Fig. 6). This effect was significantly reduced (*P* ≤ 0.002) by protease treatment of the streptococcal strain (Fig. 6A) or sialidase treatment of the PMNs (Fig. 6B); the only exception was the stimulation of sialidase-treated PMNs by *S. gordonii* SK186 (*P* = 0.318). Significant inhibition (*P* ≤ 0.005) of superoxide anion production by untreated PMNs in the presence of 5 mM sialyllactose was also observed in reaction mixtures containing six of the seven *S. gordonii* strains (Fig. 6C); the only possible exception was strain SK33 (*P* = 0.014). In contrast, superoxide production in parallel reaction mixtures was not affected by the presence of 5 mM glucuronic acid, another charged sugar, or 5 mM lactose (results not shown). Thus, the results indicate that the sialic acid-binding adhesin of each *S. gordonii* strain plays an important role in the interactions of the bacterium with human PMNs.

To further examine the biological consequence of the adhesin-mediated interaction between each *S. gordonii* strain and PMNs, reaction mixtures containing approximately 25 CFU per PMN were incubated for 30 min at 37°C, fixed, embedded, sectioned, and examined by electron microscopy. Phagocytosis of each *S. gordonii* strain was readily observed, and there were no apparent differences between strains, including the virulent strain *S. gordonii* DL1 (Fig. 7A) and the avirulent strain *S. gordonii* SK12 (Fig. 7B).

We then compared the susceptibility of each *S. gordonii* strain to the bactericidal activity of human PMNs in reaction mixtures containing cells at a constant ratio of 1 CFU to 3 PMNs (Fig. 8A). PMN-dependent killing of bacteria was not...
observed in reaction mixtures containing three of the virulent strains, \textit{S. gordonii} 38, DL1, and SK186, and was modest but significant in reaction mixtures containing each of the other two strains (42% killing of added \textit{S. gordonii} K4 \([P = 0.013]\) and 55% killing of added \textit{S. gordonii} SK33 \([P = 0.012]\)). Under identical conditions, the levels of killing of the two avirulent strains were 87% for added \textit{S. gordonii} SK9 \((P = 0.001)\) and 89% for added \textit{S. gordonii} SK12 \((P = 0.003)\). Importantly, the level of killing of avirulent strain SK9 or SK12 in these studies was significantly greater than the level of killing of virulent strain K4 or SK33 \((P = 0.001)\). In addition, killing of these four strains was not observed in reaction mixtures that contained sialidase-treated PMNs (Fig. 8A), which in previous studies (21) were shown to kill more than 90% of added \textit{A. naeslundii}.

The results of bactericidal assays performed with human

![Image](https://example.com/image1.png)
PMNs (Fig. 8A) were comparable to the results obtained with rat PMNs and selected S. gordonii strains (Fig. 8B). Thus, the numbers of virulent S. gordonii 38 or S. gordonii DL1 CFU in reaction mixtures containing rat PMNs were greater than the numbers in control reaction mixtures containing bacteria alone, while the corresponding numbers of avirulent S. gordonii SK9 and S. gordonii SK12 CFU were reduced by 70% and 84%, respectively, in the presence of rat PMNs.

**DISCUSSION**

The correlation between the infectivity of seven S. gordonii strains in catheterized rats (Fig. 1 and 3) and the ability of these bacteria to survive in PMNs following adhesion-mediated phagocytosis suggests a new and potentially important virulence determinant of infective endocarditis. Adhesion-mediated binding of each strain to PMNs stimulated superoxide anion production (Fig. 6) and triggered phagocytosis (Fig. 7), as has been observed previously for other microorganisms that recognize PMN cell surface glycoconjugates (17, 18, 21). However, the strains that caused severe endocarditis were resistant to PMN-dependent killing or the numbers of these strains were reduced only by approximately 50%, whereas the level of killing of each avirulent strain approached 90%. The differences in the resistance of virulent and avirulent S. gordonii strains to killing by PMNs were highly significant (P = 0.001) and may well be sufficient to influence the course of disease in catheterized animals. While the resistance of certain viridans group streptococci to PMN-dependent killing has been noted previously (33), the present findings provide the first evidence that we are aware of associating this property with the pathogenesis of infective endocarditis.

The predilection of virulent S. gordonii strains for damaged heart valves is evident from the severity of the infections caused by these bacteria in catheterized animals compared with the severity in uncatheterized animals (Fig. 1 and 3). Indeed, the rapid clearance of bacteria from the circulation of uncatheterized animals (Fig. 4) suggests that the shedding of virulent microorganisms from heavily infected heart valves of catheterized animals (Fig. 2B) may account for the elevated levels of bacteria in the blood, liver, and spleen (Fig. 1B). By comparison, the infections caused by S. gordonii SK9 and SK12 were minimal at best (Fig. 1, 2, and 3). Thus, while binding of bacteria to the fibrin platelet matrix or isolated platelets (Fig. 5) may be necessary for the initiation of disease, it is clearly not sufficient. A possible role for the resistance of S. gordonii to PMN-dependent killing in pathogenesis was suggested by the work of Durack (6), who examined the heart valves of catheterized rabbits 30 min after intravenous challenge of these animals with Proteus mirabilis, “Staphylococcus albus,” or a viridans group streptococcus identified as S. sanguinis serotype 2. Interestingly, most of the bacteria observed in animals infected with P. mirabilis or “S. albus” were directly attached to preexisting endocardial thrombi, whereas most of the streptococci were within PMNs or monocytes associated with such sites. It was unclear whether these streptococci attached to damaged heart valves and were rapidly phagocytosed or whether they were carried to such sites by the phagocytes. In either case, the survival of streptococci following phagocytosis may be essential for further infection resulting in dense colonies like those seen in the hearts of catheterized rats challenged with S. gordonii DL1 (Fig. 2B).

Electron microscopy of similar colonies in the heart of a catheterized rabbit (6) revealed the characteristic presence of a fibrin capsule surrounding densely packed streptococci, suggesting yet another possible mechanism by which these bacteria may evade the host defense.

The pathogenesis of infective endocarditis undoubtedly depends on a number of distinct virulence determinants. For the most part, previous investigations have focused on the contributions of specific adhesive interactions. Thus, a strain of S. sanguinis that induced the aggregation of rabbit platelets was found to be more virulent in catheterized rabbits than another strain that lacked this activity (9). In addition, the virulence of S. gordonii DL1 in catheterized rats was significantly reduced by deletion of the gene (hsa) for the sialic acid-binding adhesin of this strain (28). Importantly, sialic acid-binding adhesins occur on all S. gordonii strains included in the present investigation, supporting the notion that the differences in virulence noted among these bacteria do not depend on adhesion per se but instead depend on the ability to survive following adhesion-mediated phagocytosis. Interestingly, sialic acid-binding adhesins also occur on S. sanguinis and Streptococcus oralis (10), two other viridans group streptococci that are prominent agents of infective endocarditis (5). The fate of these bacteria following their interactions with PMNs remains to be determined.

Further studies are also needed to determine the basis for the resistance or susceptibility of different S. gordonii strains to PMN-dependent killing. It remains to be determined whether these bacteria differ in their susceptibilities to various bactericidal products of PMNs. Studies to explore this possibility may provide important clues that lead to identification of specific bacterial components associated with virulence and a starting point for identification of the corresponding genes. In such studies, the resistance of S. gordonii DL1 to PMN-dependent killing is likely to be reduced by any of several mutations that affect general cellular functions (for example, cell wall biosynthesis). Thus, a more definitive approach for identification of the genes associated with virulence may involve transfer of the genes from the virulent strain S. gordonii DL1 to an avirulent strain, such as S. gordonii SK12. Identification of genes that increase the resistance of bacteria to PMN-dependent killing could provide important insight into the pathogenesis of infective endocarditis and may suggest possible new approaches for prevention of this disease.

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