Association of Cell-Adherent Glycocalyx and Endocarditis Production by Viridans Group Streptococci

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To assess the role of glycocalyx production in the pathogenesis of endocarditis caused by viridans group streptococci in adult patients, glycocalyx production was examined for 49 blood culture isolates. The tryptophan assay, a quantitative spectrophotometric test, was used to measure cell-adherent glycocalyx production. Absorbance values of the isolates that produced endocarditis were significantly higher (means, 0.166 versus 0.060 \( [P < 0.001] \)). At a breakpoint of absorbance of 0.120, the sensitivity of the test was 0.83, the specificity was 0.96, and the predictive value was 0.95. These data suggest that the in vitro tryptophan assay of glycocalyx production by viridans group streptococci has potential value as a predictor of clinical pathogenicity.

Streptococci are the leading cause of bacterial endocarditis, accounting for about 45% of all cases. Of these, at least 75% are caused by viridans group streptococci (15). The interaction between these microorganisms and the damaged heart valve (nontuberculous endocarditis) is a crucial step in the development of endocarditis. The ability of viridans group streptococci to adhere to damaged heart valves partially explains the high incidence of endocarditis. Gould et al. demonstrated that the organisms that frequently cause endocarditis (e.g., enterococci, viridans group streptococci, Staphylococcus aureus, and Staphylococcus epidermidis) adhere to valvular endothelium significantly better than organisms that rarely cause endocarditis (5). Pelletier et al. reported that the viridans group streptococci which produce dextran (glycocalyx) have a higher likelihood of producing endocarditis in the rabbit model (11). Preincubation of these isolates with the enzyme dextranase, which digests glycocalyx, markedly inhibited the ability of these organisms to initiate the infection (1). Parker and Ball have noted that some members of the viridans group streptococcal family are more likely to be responsible for endocarditis (Streptococcus mutans, Streptococcus mitior, and Streptococcus sanguis). These organisms all produce abundant glycocalyx (10).

Studies in our laboratory have shown that the amount of glycocalyx produced is correlated with the size of infected cardiac vegetations and resistance to antimicrobial therapy (12). These relationships have commonly been measured by qualitative light microscopy assays with the polysaccharide stains ruthenium red, periodic acid-Schiff, and Cellufluor (7). To further study the role of glycocalyx in endocarditis pathogenesis, we reported a quantitative spectrophotometric assay which measures cell adherent glycocalyx produced by viridans group streptococci grown in glucose substrate (2). The assay is based on the tryptophan reaction described by Shearer et al. (13). This assay has shown correlation between the amount of cell-adherent glycocalyx, the size of the cardiac vegetation in vivo, and the ability to sterilize the streptococci with antibiotics (2). The investigation described below looks at the ability of this in vitro assay to predict the endocarditis-producing potential of blood culture isolates of viridans group streptococci.

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MATERIALS AND METHODS

Test strains. Blood culture isolates of viridans group streptococci from 49 patients were obtained from Truman Medical Center (Kansas City, Mo.) and San Francisco General Hospital (San Francisco, Calif.). Organisms were stored at \(-70°C\) in tryptic soy broth with 20% glycerol (Remel Laboratories, Lenexa, Kans.). Isolates were transferred a maximum of four times between isolation and tryptophan testing. For in vitro quantification of glycocalyx production, organisms were grown for 48 h in pooled rabbit serum (containing 75 to 76 mg of glucose per dl) as the source of carbohydrate.

Endocarditis production. One investigator reviewed the clinical records of the 49 patients and evaluated the presence of endocarditis according to the criteria established by Von Reyn et al. (14).

Quantitative (tryptophan) assay. An investigator who had no knowledge of the clinical data performed the quantitative assay on the 49 isolates. For the assay, streptococcal isolates were streaked on blood agar plates and a uniform inoculum was made in two 4-ml tubes of pooled normal rabbit serum and grown at \(37°C\) for 48 h. In daily assays, either a standard curve of 520,000-\(M_w\) dextran or a single dextran sample (0.1 mg/ml) was included as a check on run-to-run variability.

Statistical analysis. The datum group was divided into endocarditis and nonendocarditis readings on the basis of patient chart reviews, and Student's \(t\) test was used to compare the groups. A table (2 by 2) was made with summary data, and Bayes' rule was applied to assess the predictive value of the test (3).

RESULTS

Of the 49 patient isolates, 24 were judged to be endocarditis producing according to the scheme of Von Reyn et al. (14).
TABLE 1. Endocarditis-producing isolates

<table>
<thead>
<tr>
<th>Category</th>
<th>Symptoms of patient</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite</td>
<td>Histologic or bacteriologic evidence from valvular vegetation or peripheral embolus</td>
<td>4</td>
</tr>
<tr>
<td>Probable</td>
<td>Persistently positive blood cultures and new regurgitant murmur or predisposing heart disease and vascular phenomena</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Intermittently positive blood cultures, fever, new regurgitant murmur, vascular phenomena</td>
<td>8</td>
</tr>
<tr>
<td>Possible</td>
<td>Persistently positive blood cultures and predisposing heart disease or vascular phenomena</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Intermittently positive blood cultures, fever, predisposing heart disease, vascular phenomena</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>At least two positive blood cultures without an extra cardiac source, fever</td>
<td>3</td>
</tr>
</tbody>
</table>

(28). Figure 1 shows the tryptophan assay results for the 49 isolates (24 endocarditis-producing strains and 25 nonendocarditis-producing strains). The mean absorbance of the endocarditis-producing group was significantly different from that of the nonendocarditis group ($P < 0.001$). The breakpoint of $A_{500}$ (0.120) was established at $>1$ standard deviation from the means of the endocarditis (0.166 ± 0.045) and the nonendocarditis (0.060 ± 0.031) groups. Of the four patients with endocarditis and absorbance values less than 0.120, two belonged to the probable-endocarditis group and two belonged to the possible-endocarditis group. The three isolates from the possible-endocarditis group with fever and at least two positive blood cultures without an extra cardiac source (Table 1) had absorbances of 0.168 and greater. Table 2 demonstrates that the sensitivity of this assay was 0.83, the specificity was 0.96, and the predictive value was 0.95.

**DISCUSSION**

It is often difficult to correlate a positive blood culture with clinical pathogenicity. The physician has to decide whether the culture denotes infection, spontaneous bacteremia, or blood culture contamination. This is a larger problem in patients with underlying diseases for which clinical presentations may be atypical and in hospitalized patients who have undergone multiple invasive procedures. Laboratory markers can often aid in deciding the pathogenic potential of a blood culture isolate. Exopolysaccharide (slime) production of *S. epidermidis* has often correlated with pathogenicity (6). The presence of teichoic acid antibody is advocated by some to correlate with serious *S. aureus* infections (9).

Finding viridans group streptococci in the blood alerts the physician to the possibility of bacterial endocarditis. It is estimated that between 12 and 60% of patients with blood cultures positive for these organisms will have bacterial endocarditis (8). This likelihood increases when there is underlying valvular heart disease (congenital or rheumatic). Some species of viridans group streptococci are more often responsible for endocarditis. In fact, blood cultures positive for *S. mutans* indicate endocarditis >90% of the time (10). Unfortunately, it is extremely difficult for most laboratories to identify viridans group streptococci reproducibly. Some authorities report that it is not appropriate to even attempt this (4) because of variations among laboratories. The tryptophan assay may alleviate some of these problems. An isolate of viridans group streptococci with an absorbance of >0.120 has a high likelihood of causing endocarditis. For a given sensitivity and specificity, the predictive ability of a test decreases with decreasing prevalence of the disease. This is important for infrequent diseases like endocarditis, even with highly sensitive and specific tests. We recognize that the sensitivity and specificity data for the series of patients used in this study may not be as statistically

**TABLE 2. Correlation: tryptophan assay of glyocalyx production and endocarditis occurrence**

<table>
<thead>
<tr>
<th>$A_{500}$ test result</th>
<th>With endocarditis</th>
<th>Without endocarditis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (&gt;0.120)</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Negative (&lt;0.120)</td>
<td>4</td>
<td>24</td>
</tr>
</tbody>
</table>
valuable when the prevalence of endocarditis in the general tested population is considered. This should not be interpreted, however, as lessening the potential of this assay. Prospective evaluation and some automation of this procedure will determine the utility of this assay in predicting the virulence of individual isolates of viridans group streptococci.

The correlation of the cell-adherent polysaccharide with clinical pathogenicity gives a rationale for further investigating the character and nature of this bacterial glycocalyx.

LITERATURE CITED


