Teichoic Acid Antibody Determination by Agar-Gel Diffusion:
Effect of Using Dilute Antigen Preparations

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Because the reported frequency of teichoic acid precipitins in controls and various patient groups has varied considerably among laboratories, we studied the effect of using various concentrations of staphylococcal extracts in agar-gel diffusion tests for teichoic acid antibodies. Of 25 normal sera, only 1 was positive against an undiluted extract, but 4 were positive against a 16-fold-diluted extract. Of nine sera from patients with staphylococcal bacteremia, two were positive at a higher titer against the diluted extract. A false-positive serum against the undiluted extract had a twofold titer increase against the diluted extract. Because human immune serum globulin is generally used as a positive teichoic acid antibody control, the variability of five different lots was studied. Three lots had teichoic acid antibody titers of 1:4, whereas each had titers of 1:2 and 1:8. Based on this study, we feel that staphylococcal extracts should not be diluted. If immune serum globulins are used to determine the adequacy of ultrasonic extracts, newly acquired globulin lots should be standardized against an ultrasonic extract of proven sensitivity and specificity.

Teichoic acid antibody assays have become important aids for diagnosing serious staphylococcal infections (1, 3, 6, 8, 10, 11, 13). Although most laboratories report using ultrasonic extracts prepared by the method of Crowder and White, few describe standardization of the methodology. This lack of standardization might explain discrepancies in the reported incidence of true-positive results in infected patients and false-positive results in patients without staphylococcal infection or in normal persons. Antigen concentration is one factor which could affect assay sensitivity. In this report we show the effect of using dilute antigens on the sensitivity of agar-gel diffusion tests for teichoic acid antibodies.

MATERIALS AND METHODS

Agar-gel diffusion. Ultrasonic extracts of the Lafferty strain of Staphylococcus aureus were prepared as previously described (2). Sera and antigens were diluted in 0.15 M sodium chloride and tested by agar-gel diffusion (9). Commerically available human immune serum globulin, Gammagee (Merck Sharp & Dohme, West Point, Pa.), was tested as a positive teichoic acid antibody control. Sera and Gammagee were tested against serial dilutions of the extract.

RESULTS

Characterization of the ultrasonic extract. The ultrasonic extract contained 5.5 mg of protein per ml by the Lowry method (5); protein accounted for 40% of the dry weight of the extract. The extract contained 1.8 mg of nucleic acid per ml, as determined by comparison of the optical density at 260 and 280 nm (12). Gammagee, lot 0695A, had a 1:4 teichoic acid antibody titer against the undiluted extract. The teichoic acid precipitin line seen with our extract formed a line of identity with authentic ribitol teichoic acid provided by G. W. Ross, Glaxo Research Ltd., Greenford, Middlesex, London, England. The teichoic acid precipitin line split into two lines at extract dilutions greater than 1:8.

Effect of dilution of the ultrasonic extract on teichoic acid antibody titers. Twofold dilutions of Gammagee and sera from two normal persons were tested against serial dilutions of the ultrasonic extract. The teichoic acid antibody titer of Gammagee and the two normal sera increased when dilute ultrasonic extracts were used (Table 1). The importance of the increased sensitivity observed with the dilute extract was evaluated by testing sera from normal persons without any clinically recognized recent staphylococcal infection and patients with staphylococcal infections. Sera from 25 healthy controls, 9 patients with staphylococcal bacteremia, and 1 patient without recent staphylococcal infection whose undiluted serum was previously found to be falsely positive for tei-
Teichoic acid antibodies were tested against undiluted and 16-fold diluted staphylococcal ultrasonic extracts. Of the 25 normal sera, 1 was positive against the undiluted extract and 4 were positive against the 16-fold diluted extract; all four normal sera were positive at titers of undiluted extract only. Teichoic acid antibody titers of the ten patients are shown in Table 2. Two sera were positive only against the diluted extract, and two others were positive at higher titers against the diluted than the undiluted extract. The single false-positive serum tested was positive at a 1:2 serum dilution against the diluted extract. Thus, dilution of the extract results in an increased frequency of false-positive results in normals and increased teichoic acid antibody titers in patients with staphylococcal infection or false-positive results.

Ouchterlony plates were examined at 18 and 24 h; the optimal incubation period was 18 h for undiluted and 24 h for diluted extracts. Precipitin lines which were seen at 18 h of incubation against undiluted extracts had disappeared by 24 h for three sera. Also, precipitin lines not seen at 18 h were visible at 24 h of incubation for six sera tested against diluted extracts.

Comparison of production lots of human immune serum globulins. Five immune serum globulin preparations were examined: Gammage lots 0695A, 1255W, 2865W, and 1633A, and Immun-G (Parke, Davis & Co., Detroit, Mich.) lot 914684A. The teichoic acid antibody titers of the immune serum globulin preparations were 1:8, 1:4, 1:2, 1:4, and 1:4, respectively, against the undiluted ultrasonic extract. The teichoic acid precipitin titers of the ultrasonic extract against the undiluted immune serum globulin preparations were 1:2,048, 1:1,024, 1:64, 1:2,048 and 1:512, respectively. Thus, lot differences among immune serum globulin preparations resulted in variable teichoic acid precipitin titers of the ultrasonic extract.

**DISCUSSION**

Assays for teichoic acid antibodies are useful for the rapid and specific diagnosis of staphylococcal endocarditis (1, 8, 10) and for distinguishing complicated from uncomplicated bacteremia (11, 13). Counterimmunoelectrophoresis, although more rapid than agar-gel diffusion, may be associated with false-positive results in up to 57% of normal persons (4). Although we have found a radioimmunoassay to be superior to agar-gel diffusion (13), radioimmunoassay requires special equipment and is not available in most laboratories. Thus, agar-gel diffusion is the most widely applicable method for measuring teichoic acid antibodies.

Most studies using agar-gel diffusion report that sera from 90% of patients with severe staphylococcal infections such as endocarditis contain teichoic acid antibodies (1, 8, 13). Although most normal persons are shown to have antibodies to staphylococcal ribitol teichoic acid when more sensitive methods such as double diffusion in tubes or radioimmunoassay are used (2, 13), less than 10% of normals are shown to have teichoic acid antibodies by agar-gel diffusion techniques (1, 8, 13). Positive results in normal persons or patients without staphylococcal infections represent false-positive results to clinicians who are

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**TABLE 1. Effect of dilution of the staphylococcal extract on teichoic acid antibody titers**

<table>
<thead>
<tr>
<th>Extract dilution</th>
<th>Gammage(a)</th>
<th>Normal no. 1</th>
<th>Normal no. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>1:4</td>
<td>N</td>
<td>U</td>
</tr>
<tr>
<td>1:2</td>
<td>1:4</td>
<td>N</td>
<td>U</td>
</tr>
<tr>
<td>1:4</td>
<td>1:4</td>
<td>N</td>
<td>1:2</td>
</tr>
<tr>
<td>1:8</td>
<td>1:8</td>
<td>N</td>
<td>1:2</td>
</tr>
<tr>
<td>1:16</td>
<td>1:8</td>
<td>U</td>
<td>1:2</td>
</tr>
</tbody>
</table>

\(a\) N, Negative; U, undiluted.

\(b\) Commercial human immune serum globulin (lot no. 0695A; Merck Sharp & Dohme).

\(c\) Highest serum dilution positive for teichoic acid antibodies.

**TABLE 2. Effect of dilution of the staphylococcal extract on teichoic acid antibody titers in nine patients with staphylococcal infections and one patient with a false-positive result**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Diagnosis</th>
<th>Teichoic acid antibody titers(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Undiluted extract</td>
</tr>
<tr>
<td>1</td>
<td>Bacteremia(c)</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>Endocarditis</td>
<td>1:2</td>
</tr>
<tr>
<td>3</td>
<td>Endocarditis</td>
<td>U</td>
</tr>
<tr>
<td>4</td>
<td>Endocarditis</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>Bacteremia</td>
<td>1:2</td>
</tr>
<tr>
<td>6</td>
<td>Bacteremia</td>
<td>U</td>
</tr>
<tr>
<td>7</td>
<td>Endocarditis</td>
<td>1:2</td>
</tr>
<tr>
<td>8</td>
<td>Bacteremia</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>Endocarditis</td>
<td>1:4</td>
</tr>
<tr>
<td>10</td>
<td>False positive</td>
<td>U</td>
</tr>
</tbody>
</table>

\(a\) Values indicate highest serum dilution positive for teichoic acid antibodies. N, Negative; U, undiluted.

\(b\) Extract was diluted 16-fold in 0.15 M saline.

\(c\) Uncomplicated bacteremia (i.e., no evidence of endocarditis or metastatic infection). Other patients with staphylococcal infection had either endocarditis or complicated bacteremia except for patients no. 6 and no. 8 who had suppurative at intravascular access sites which had to be excised to control the infection. Patient no. 10 was undergoing routine preoperative evaluation for jejuno-ileal bypass and gave no history of staphylococcal infection.
trying to identify patients with serious staphylococcal infections. We have demonstrated the specificity of our assay using undiluted extracts by showing that only a small proportion of normal persons or patients with non-staphylococcal infections demonstrate teichoic acid precipitins (1, 13). Antibody titers in patients with endocarditis and frequencies of false-positive results vary considerably in reported studies (3, 4, 13). Conceivably, this variability could result from (i) strain differences of organisms used to prepare extracts, (ii) different extraction methods, (iii) different agar-gel diffusion methods, or (iv) any combination of i, ii, and iii.

Leffell and co-workers, using an antigen adjusted to a protein concentration of 1 mg/ml, reported positive results in over 25% of normals, occasionally at titers as high as 1:2 (4). Their extract was more dilute than ours (5.5 mg of protein per ml). As noted in this study, false-positive antibody results are more common when diluted extracts are used; thus, the high frequency of false-positive results in their study may have resulted from use of a diluted extract. They used a diluted extract to eliminate the protein A precipitin line to facilitate identification of the teichoic acid line. However we have not found that protein A precipitins interfere with the recognition of teichoic acid precipitins.

Diluting antigens is known to increase the sensitivity of double-diffusion tests (7). The visible precipitin line which forms at the zone of antigen and antibody equivalence migrates toward the antigen well as the antigen is diluted (7). Presumably the zone of equivalence occurs in the antibody well when normal sera are tested against undiluted staphylococcal extracts. As the extract is diluted, the zone of equivalence migrates into the agar between the wells, explaining the false-positive results in normal persons and the higher-titer results in patients with serious staphylococcal infections.

Other factors may have contributed to the high incidence of false-positive results in the study by Leffell and co-workers. They used agarose, whereas we used Noble agar. Others have reported an 18% incidence of false-positive results with agarose (3).

Based on our experience, as exemplified by the data in this report, we would not recommend diluting staphylococcal extracts when prepared by the method Crowder and White (1). We had hoped that titration of newly prepared extracts against immune serum globulin might be a simple method for determining whether the extract needed dilution, concentration, or repeat preparation. However, different production lots of globulin from the same manufacturer have different amounts of teichoic acid antibodies; therefore, this technique cannot be used unless a single lot of immune serum globulin is used by all investigators comparing results. Ideally, all immune serum globulins and staphylococcal extracts should be standardized against a single extract or a single immune serum globulin lot which are of known sensitivity and specificity from previous testing of large groups of patients and normal controls.

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LITERATURE CITED