Teichoic Acid Antibodies in Chronic Staphylococcal Osteomyelitis

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Gel-diffusion and the enzyme-linked immunosorbent assay (ELISA) were used to quantify and to identify the immunoglobulin class of teichoic acid antibodies in patients with chronic staphylococcal osteomyelitis and a wide variety of other infections. Teichoic acid antibodies were identified by gel-diffusion in 14 of 23 patients with staphylococcal endocarditis, six of 30 with staphylococcal bacteremia without endocarditis, four of 35 with staphylococcal skeletal infections, and one of 45 with monostaphylococcal infections. None of the 20 patients with chronic staphylococcal osteomyelitis had positive gel-diffusion assays, even though many had had their infections for several years. The ELISA method was more sensitive than gel-diffusion in measuring teichoic acid antibodies, but was also much less specific. Teichoic acid antibodies were detected predominantly in the IgG fraction of serum. Our findings suggest that the presence and degree of antigenemia are more important than the duration of the staphylococcal infection in stimulating production of teichoic acid antibodies.

Teichoic acids are the major group antigens of Staphylococcus aureus (1). High titers of antiteichoic acid antibodies have been shown by gel-diffusion or counterimmunoelectrophoresis, or both, in most patients with staphylococcal endocarditis, as well as in many patients with other bacteremic staphylococcal infections (2-7). However, the prevalence of teichoic acid antibodies in nonbacteremic staphylococcal infections, such as chronic staphylococcal osteomyelitis is not known. Because cultures of operative specimens are generally required to establish a bacteriologic diagnosis in patients with chronic osteomyelitis (8), a reliable serologic test could greatly simplify the process of establishing the bacterial cause of chronic bone infections. Therefore, we used the gel-diffusion assay and the highly sensitive enzyme-linked immunosorbent assay (ELISA) to measure teichoic acid antibodies in patients with staphylococcal skeletal infections and a variety of other infections. We also used the same assays to identify the immunoglobulin class of these antibodies.

Methods

PATIENTS

Thirty-five patients with staphylococcal skeletal infections were studied. Ten had acute osteomyelitis, 20 had chronic osteomyelitis, and five had acute arthritis. Nine of the 10 patients with acute staphylococcal osteomyelitis and four of the five patients with acute arthritis had associated staphylococcal bacteremia. A bacteriologic diagnosis was established in patients with chronic staphylococcal osteomyelitis by culture of a bone biopsy specimen or an aspirate of loculated pus (13 patients), by repeated isolation of S. aureus from blood cultures (four patients), or by isolation of S. aureus from blood cultures obtained during an earlier episode of acute osteomyelitis (three patients). The duration of chronic osteomyelitis in these patients varied from 1 month to 26 years (average, 6.9 years).

Fifty-three patients with other bacteremic staphylococcal infections were examined. Twenty-three had a clinical diagnosis of endocarditis (three confirmed pathologically), seven supplicative phlebitis, 10 presumed shunt infections (that is, chronic hemodialysis patients with unexplained staphylococcemia), and 12 transient bacteremia either in association with a staphylococcal carbuncle or without apparent source. Forty-five patients with various infections caused by organisms other than staphylococci were also examined.

ANTIGEN PREPARATION

Teichoic acid antigen was prepared according to the method of Crowder and White (3). Lafferty strain S. aureus (coagulase positive, phage group 52/423/80/81) was harvested with normal saline after overnight growth at 37°C on pans containing trypticase soy agar. Antigen extracts were prepared by subjecting a 20% (vol/vol) suspension of organisms to ultrasonic vibration for 5 h in a Branson Sonifer cell disrupter 185 (Heat Systems—Ultrasonics, Plainview, New York), cooled with circulating water at 4°C. Supernatant fluid obtained after centrifugation at 15 000 g for 30 min was stored at −20°C until used as antigen in gel-diffusion studies. Purified teichoic acids with both alpha- and beta-N-acetylglucosaminyl ribitol teichoic acids were provided by Dr. G.W. Ross (Glaxo Laboratory, Greenford, England).

GEL-DIFFUSION STUDIES

Ouchterlony plates composed of 1.5% agarose in phosphate buffer, pH 8.0, (Meloy, Springfield, Virginia), were used in these studies. Each plate contained six circular wells (one central and five peripheral). The staphylococcal sonicate was placed in the central well. A positive and a negative control serum were placed in two of the peripheral wells and serial twofold dilutions of the test serum in 0.9 N saline in the remaining three wells. The plates were examined for teichoic acid precipitin bands after incubating at 37°C for 6, 18, 48, and 72 h in a humid chamber.

ELISA STUDIES

Antibody to purified teichoic acid was quantified using the ELISA method (9). Polystyrene plastic tubes were coated with purified teichoic acid at a concentration of 50 µg/ml because this was the lowest concentration that produced an optical density greater than 1.0 with a 1:100 dilution of hyperimmune serum when tested with goat antihuman IgG. Dilutions of serum were added, and antigen-antibody complexes were detected with alkaline phosphatase conjugates of goat antihuman IgG,
IgM, or IgA by examining the tubes spectrophotometrically as previously described (10).

CHARACTERIZATION OF IMMUNOGLOBULIN CLASS

Both gel-diffusion and the ELISA method were used to measure specific antibody in gel-diffusion-positive and -negative serum specimens that had been separated previously into fractions of differing immunoglobulin classes. Five millilitres of serum were first added to a Sephadex G-200 (Pharmacia Fine Chemicals, Inc, Piscataway, New Jersey) column equilibrated with 0.1 M phosphate buffer, pH 6.8, and containing 0.02 M sodium azide. Fractions were concentrated to an equivalent initial volume, and analyzed for immunoglobulin class and antibody titer.

Results

Teichoic acid antibodies were detectable by gel-diffusion in four of 35 patients with staphylococcal skeletal infections; however, each of these four patients had concomittant staphylococcal bacteremia (Table 1). No patient with chronic staphylococcal osteomyelitis had a positive gel-diffusion test. Although 14 of 23 patients with a clinical diagnosis of endocarditis had teichoic acid antibodies detectable by gel-diffusion, none of the three patients with pathologically confirmed staphylococcal endocarditis had positive gel-diffusion studies. One patient had both staphylococcal endocarditis and chronic staphylococcal osteomyelitis. Teichoic acid antibodies were detectable at a titer of 1:2 during the episode of endocarditis, but fell to undetectable levels after successful treatment of the endocarditis, in spite of progressive, chronic staphylococcal osteomyelitis.

Positive gel-diffusion tests were present in six of 30 patients with staphylococcal bacteremia without evidence of endocarditis and were most common in patients with presumed shunt infections (four of 10) and suppurative phlebitis (two of seven). Only one patient with an infection involving another organism other than S. aureus had a positive gel-diffusion test. This patient had cellulitis with associated Streptococcus pyogenes bacteremia.

Antibodies to teichoic acid were identified by gel-diffusion in fractions that contained IgG but not in fractions containing IgA or IgM. Analysis of serum specimens for teichoic acid antibodies in the three Ig classes by the ELISA method showed IgG antibody in all patients, as opposed to IgM antibody in less than half and IgA antibody in none. Titers were highest in gel-diffusion-positive patients with staphylococcal infections and were progressively lower in gel-diffusion-negative patients with staphylococcal infections, patients with nonstaphylococcal infections, and normal control subjects (Table 2).

Discussion

These studies establish the immunoglobulin class of teichoic acid antibodies as IgG and show that when a highly sensitive assay such as the ELISA is used, teichoic acid antibodies can be found in the sera of most persons. However, only patients with bacteremic staphylococcal infections had titers of sufficient magnitude to be detectable by gel-diffusion. Teichoic acid antibodies were not detectable by gel-diffusion in patients with nonbacteremic staphylococcal infections such as chronic staphylococcal osteomyelitis.

Chronic staphylococcal osteomyelitis fails to induce teichoic acid antibodies in sufficiently high titer to be demonstrable by gel-diffusion, even when the infection has been present for many years. This relatively poor antibody response in patients with staphylococcal osteomyelitis has previously been observed and attributed to the low level of staphylococcal antigenemia present in these patients (6). In light of our data, the presence and the degree of antigenemia would appear to be more important in stimulating teichoic acid antibody production than the duration of the staphylococcal infection.

Although teichoic acid antibodies could be shown in patients with chronic staphylococcal osteomyelitis when the ELISA was used to analyze sera, patients with infections caused by organisms other than S. aureus and normal control subjects also had positive ELISA findings. Similar results have been obtained when Preer gel-diffusion tubes have been used to assay teichoic acid antibodies (2). Thus, if a sensitive technique such as the ELISA is used, teichoic acid antibodies can be found in most persons. Although higher ELISA titers were observed in patients with chronic staphylococcal osteomyelitis than in patients with nonstaphylococcal infections or normal

Table 1. Gel-Diffusion Teichoic Acid Antibody Studies in Patients with Staphylococcal and Nonstaphylococcal Infections

<table>
<thead>
<tr>
<th>Infection</th>
<th>Total</th>
<th>Positive*</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal infections</td>
<td>35</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Acute osteomyelitis</td>
<td>10</td>
<td>3†</td>
<td>30</td>
</tr>
<tr>
<td>Chronic osteomyelitis</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arthritis</td>
<td>5</td>
<td>1†</td>
<td>20</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>23</td>
<td>14</td>
<td>61</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>30</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Presumed shunt infection</td>
<td>10</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>Suppurative phlebitis</td>
<td>7</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>Carbuncle or spontaneous</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nonstaphylococcal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal infections</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Endocarditis</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>23</td>
<td>1†</td>
<td>4</td>
</tr>
</tbody>
</table>

* All gel-diffusion-positive sera were titered. Titers of ≥ 1:4 were found most often in patients with staphylococcal endocarditis. However, occasionally patients with staphylococcal bacteremia without evidence of endocarditis also had high titer.

† Concomitant Staphylococcus aureus bacteremia.

† Staphylococcal cellulitis with associated bacteremia.

Table 2. IgG Anti-Teichoic-Acid Titers in Gel-Diffusion-Positive Patients (A), Gel-Diffusion-Negative Patients with Staphylococcal Infections (B), Patients with Nonstaphylococcal Infections (C),

<table>
<thead>
<tr>
<th>Group</th>
<th>IgG Anti-Teichoic-Acid Titers*</th>
<th>P Value †</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
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</tbody>
</table>

* Log₁₀ ± sa.

† Different from Group A as measured by t-test (Fisher and Yates).

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control subjects, there was considerable overlap among the groups.

Teichoic acid antibodies were detectable by gel-diffusion in only 61% of our patients with endocarditis. This rate is lower than the rates of 85% to 100% previously reported (3-7). Several explanations for the difference are possible. In our series the diagnosis of endocarditis was a clinical one and, as such, subject to error. Although some of the patients included in this group may, in fact, have had no endocardial infection, the three patients with pathologically confirmed staphylococcal endocarditis and negative gel-diffusion studies argue against this as the sole explanation for the relatively high prevalence of gel-diffusion-negative patients in our endocarditis group. These three gel-diffusion-negative patients with pathologically confirmed endocarditis were examined at 21, 8, and 6 days respectively, after the onset of their symptoms. Because antibodies are occasionally not detectable in patients with endocarditis until 4 to 10 days after the onset of symptoms (3, 6, 7), it is possible that some patients with negative findings might have had positive gel-diffusion studies if re-examined at a later time. Finally, previous studies of teichoic acid antibodies in endocarditis have dealt almost exclusively with drug abusers. Only six of our 24 patients were recognized drug abusers. Five of these had positive gel-diffusion tests, and the sixth patient was examined only 24 h after the onset of symptoms. In contrast, only nine of 17 non-drug abusers with staphylococcal endocarditis had positive tests. Although not statistically significant, this difference suggests that drug abusers with staphylococcal endocarditis might represent a subgroup within the total population of patients with staphylococcal endocarditis having unusually high titers of teichoic acid antibodies, perhaps because of repeated antigen stimulation associated with nonsterile intravenous injections.

The sensitivity and specificity of teichoic acid antibody assays as tests for staphylococcal infections vary dramatically with both the type of assay used and the kind of staphylococcal infection being evaluated. The gel-diffusion assay is highly specific, because, except for patients with staphylococcal infections, only occasional patients with streptococcal infections (our single false-positive case) and diphtheroid endocarditis have positive results (11). Its specificity makes it a useful test for evaluating patients whose cultures are invalid because of previous antibiotic therapy. Unfortunately, gel-diffusion is a relatively insensitive assay, even in staphylococcal endocarditis, because non-drug abusers and patients seen early in the course of their infection may have negative assays at the time of their initial examination. The gel-diffusion assay is an even less sensitive test for bacteremic staphylococcal infections other than endocarditis and is a totally insensitive test for chronic staphylococcal osteomyelitis (a nonbacteremic infection). Although the ELISA is much more sensitive than gel-diffusion, it is also much less specific, and therefore offers little as a diagnostic test for staphylococcal osteomyelitis (a nonbacteremia infection).

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