Discrepancy Between Results of the Streptozyme Test and Those of the Antideoxyribonuclease B and Antihyaluronidase Tests

BENGT HEDERSTEDT,*† STIG E. HOLM,* and TORKEL WÅSTROM*†

Department of Bacteriology, National Bacteriological Laboratory, S-10521 Stockholm,† Department of Clinical Bacteriology, University of Umeå, S-901 85 Umeå,* and Department of Bacteriology, Royal Veterinary College, Biomedicum, Box 583, S-751 23 Uppsala,† Sweden

Received for publication 22 May 1978

Comparison of the serum titers obtained with the Streptozyme, the antistreptolysin O, the antideoxyribonuclease B, and the antistreptohyaluronidase tests suggested that the Streptozyme test had failed to detect antibodies against streptococcal deoxyribonuclease B and hyaluronidase. Moreover, sera that were negative in the Streptozyme test could be shown by immunodiffusion to possess significant numbers of precipitins against extracellular factors produced by group A streptococci. Follow-up studies on patients with diagnosed streptococcal infections revealed elevated antideoxyribonuclease and streptohyaluronidase titers and increased numbers of precipitation lines without simultaneous increased titers by the Streptozyme test. There is thus a need for stricter control of possible batch-to-batch variations and more careful standardization of the antigen content of the Streptozyme test.

Following an infection with group A beta-hemolytic streptococci, antibodies against extracellular and somatic antigens can be demonstrated in the serum of the patient. Serological tests for routine diagnostic purposes have been developed for the detection of antibodies mainly against extracellular antigens (11). In the past, most laboratories have used only one serological test. The most frequently performed has been the antistreptolysin O (ASO) reaction introduced by Todd (22).

In view of all the accumulated information about the variability of antibody response in streptococcal infections due not only to the localization of infection (9, 10) but also to quantitative as well as qualitative differences in extracellular antigens produced by different strains, the use of only one reaction can hardly be accepted in modern diagnosis of streptococcal infection.

The commercial availability of the Streptozyme test (8), an easily performed, single-step hemagglutination test said to detect antibodies to five different streptococcal exoproteins, namely streptolysin O, deoxyribonuclease B (DNase B), hyaluronate lyase (hyaluronidase), streptokinase, and nicotinamide adenine dinucleotide glycohydrolase, was therefore received with great interest. Several reports have confirmed the usefulness of the Streptozyme test in comparison with the ASO and anti-DNase B (ADNase B) tests (2, 4, 8, 13, 14, 16, 17). The results of the present study suggest, however, that with the antigen batch(es) used the Streptozyme test failed to detect antibodies against streptococcal DNase B and hyaluronidase.

**MATERIALS AND METHODS**

**Sera.** Three categories of human sera were tested. (i) Sera from healthy Swedish blood donors. The sampling dates of the sera were evenly distributed throughout the year. (ii) Sera sent to the laboratory for testing for streptococcal antibodies. No information on the clinical history of the patients was obtained. (iii) Selected sera from patients with known history of disease. The patients were followed serologically by repeated sampling.

**ASO.** The ASO test was performed according to the macromethod described by Ipsen (7). In each test, the titers of three human control sera, which had been adjusted to the World Health Organization international standard serum, were determined. A titer of >200 IU/ml was regarded as elevated (7).

**ADNase B.** The ADNase B test was performed by the methyl-green microtechnique of Nelson et al. (15). Serum dilution increments of 0.30 log were utilized. The streptococcal DNase B was prepared and purified by isoelectric focusing as described by Smyth and Fehrenbach (20) and Wadstrom et al. (23). No differences in ADNase tests were recorded between the use of this antigen, an antigen received from Beckman Instruments, Inc. (Fullerton, Calif.), and an antigen received through the courtesy of J. Widdowson, Colindale, England. In each test the titers of three human control sera were determined. The ADNase titers of these sera had been previously adjusted with respect to reference sera derived from Colindale and Beckman.
Instruments, Inc. A titer of >400 was regarded as elevated.

AH. For the antistreptohyaluronidase (AH) test, a modification of the mucin clot prevention technique (6) was employed using a commercially available reagent (Bacto-AHT Kit; Difco Laboratories, Detroit, Mich.; batch no. 618839). The excess of hyaluronidase is measured by its ability to hydrolyze potassium hyalurate. In each test the titers of a serum control from Difco Laboratories and a serum from a Swedish blood donor were determined. A titer of >256 was regraded as elevated (18).

Streptozyme test. The reagent (Wampole Laboratories, Dist., Stamford, Conn.) consisted of formaldehyde-treated sheep erythrocytes sensitized with group A streptococcal extracellular products. The batch numbers of the reagents used were St-83 and St-87. The test was performed according to the instructions of the manufacturer. Sera were diluted from 1:100 in a twofold dilution series. With each test, one negative and one positive serum control (Wampole Laboratories) were titered. A titer of ≥100 was regarded as positive.

ID. Immunodiffusion (ID) analyses were made according to the method described by Wadsworth (24). The ID plates were incubated at 22°C for 3 days, washed, dried, and stained with Coomassie brilliant blue. All test results were recorded from the stained plates. Sera were tested against a concentrated culture filtrate of streptococcal strain S84 type 3 known to produce streptolysin O, hyaluronidase, streptokinase, DNase, nicotinamide adenine dinucleotide glycodrolase, erythrogenic toxin, and proteinase precursor.

The average number of precipitation lines in analyses of sera from adults without recent streptococcal infections and with ASO titers of ≤200 is four to five (5).

RESULTS

Normal titers. The ASO and ADNase B titers of sera from 200 healthy blood donors were determined. Fifty of the sera were also tested by the Streptozyme test. When the upper limits of normal titers were taken as the titers exceeded by no more than 15% of a normal population (12), ASO titers of >200 and ADNase titers of >400 were considered as elevated in the present study (Table 1). By the Streptozyme test, 26% of the sera tested had a titer of ≥100.

Discrepancies between ASO and ADNase titers. Sera from 682 patients sent for routine analyses for streptococcal antibodies were tested by the ASO and ADNase tests. ADNase titers of >400 combined with ASO titers of ≤200 were found in 157 out of 682 sera, i.e., 23% (Table 2).

Thirty out of the above 157 sera were selected, such that the differences between the values of the ADNase and the ASO titers were at least three dilution increments (0.5 log units) for each serum. The ADNase and ASO titers of the 30 sera ranged from 800 to 12,000 and <50 to 200, respectively. These selected sera were further tested by the AH, Streptozyme, and ID tests. By the ID test, 29 sera showed five or more precipitation bands, and by the AH test, 22 out of 29 sera tested were found positive. By the Streptozyme test, however, only eight sera turned out to be positive (Table 3). Discrepancies between results obtained by the ADNase, the AH, and the ID tests on the one hand and the ASO and the Streptozyme test on the other hand were also found upon serological follow-up studies on selected patients. Typical serological findings with one such patient are recorded in Table 4. A few days after the 60-year-old patient showed symptoms of disease (erysipelas), group A streptococci were demonstrated and serological examination revealed low titers by all the tests. Four weeks afterwards, significant increases in titers were demonstrated by the ADNase and AH tests. Three additional precipitation lines were found by the ID test. The result of the Streptozyme test, however, remained negative, and there was an increase in ASO titer from 50 to 200.

Table 1

<table>
<thead>
<tr>
<th>Titer</th>
<th>ASO</th>
<th>ADNase</th>
<th>Streptozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;100</td>
<td>25</td>
<td>19</td>
<td>74</td>
</tr>
<tr>
<td>100</td>
<td>37</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>200</td>
<td>26</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>400</td>
<td>11</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>≥800</td>
<td>1</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>ASO titer</th>
<th>No. of sera having ADNase titer:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;400</td>
</tr>
<tr>
<td>&gt;200</td>
<td>60</td>
</tr>
<tr>
<td>≤200</td>
<td>157</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Test titers</th>
<th>No. of sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH</td>
<td></td>
</tr>
<tr>
<td>&lt;256</td>
<td>7</td>
</tr>
<tr>
<td>≥256</td>
<td>22</td>
</tr>
<tr>
<td>Streptozyme</td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>22</td>
</tr>
<tr>
<td>&gt;100</td>
<td>8</td>
</tr>
<tr>
<td>ID*</td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>1</td>
</tr>
<tr>
<td>≥5</td>
<td>29</td>
</tr>
</tbody>
</table>
DISCUSSION

The ADNase B test was adopted in Sweden in 1973. At about the same time, the Streptozyme test was introduced on the European market. Before 1973 the ASO test was the only serological reaction routinely used in Sweden for the diagnosis of streptococcal infections. Comparative serological studies with the three tests, Streptozyme, ADNase B, and ASO, were thus of interest. The DNase B antigen was locally prepared because no antigen was commercially available in Europe. The analysis of sera from healthy adult blood donors revealed that the upper limit of normal titers for ADNase B was 400, a significantly higher value than 120, the corresponding value reported from the U.S.A. (12), and the titer of 240 reported from West Germany (14). This high "normal ADNase value" found in the Swedish population is in agreement with the findings by Coburn and Pauli (3) and Rantz et al. (19) that ASO titers are higher in northern latitudes.

A comparison of results of routine analyses of sera from unselected patient material (no information was obtained on the background of the patients’ diseases) by ADNase B and ASO tests revealed the occurrence of a great number (23%) of sera with positive ADNase B and negative ASO tests (Table 2). To test the specificity of the ADNase B test, 30 sera with high ADNase B and low ASO titers were selected and tested by the Streptozyme test. This test was said to be at least as sensitive and specific as the ASO and ADNase tests. The Streptozyme test was negative in 22 out of the 30 selected sera, a result suggesting either that the Streptozyme test had not detected the increased ADNase titers, or, which is most unlikely, that the elevated ADNase titers were false positive.

A previous study (5) had shown that sera from adults without recent streptococcal infections and with an ASO titer of <200 when analyzed in ID against an extracellular streptococcal preparation gave up to five immunoprecipitates. The antibody responses in terms of the precipitation patterns produced by the 30 above-mentioned sera by ID and of the AH test were compared with the results obtained by the Streptozyme test. In 29 out of the 30 sera with high ADNase titers, ≥5 precipitation lines were demonstrated. The antigen preparation used for ID tests contained all five streptococcal factors purported to be present on the erythrocytes in the Streptozyme test. Although no attempt was made in this study to identify the nature of the streptococcal antigens with which the human precipitins reacted, it can be reasonably claimed that ID in all probability detected increased antibody titers to antigens that could be present in the Streptozyme test, either those specified by the manufacturer or others present as a result of coupling a crude antigen mixture to treated erythrocytes. Moreover, the majority of sera tested had elevated AH titers.

These findings strengthened the suggestion that the Streptozyme test had failed to detect antibodies against streptococcal DNase B and hyaluronidase. Further support for this conclusion was given by results of serological follow-up studies on patients with diagnosed streptococcal infections. Elevated ADNase and AH titers were recorded in patients’ sera, and the number of precipitation lines increased after infection without a simultaneous demonstration of increased titers by the Streptozyme test.

According to the manufacturer, the blood cells in the Streptozyme test are coated with five different antigens—there are probably others too—but the concentration of the antigens cannot separately be controlled and might vary from batch to batch.

The advantage of simultaneous determination of antibodies to different streptococcal extracellular antigens has been known for a long time (1, 21). Thus, with a battery of tests elevation of the titer of antibodies to at least one antigen can be found in almost all patients with rheumatic fever and acute glomerulonephritis. The performance of many different tests would, however, be too expensive for most laboratories. A Streptozyme test living up to the manufacturer’s claims would no doubt be a most desirable screening test for the serological diagnosis of
streptococcal infections. To reach such a standard for the Streptozyme test, the reagent used should most likely consist of a mixture of blood cells separately sensitized with standardized amounts of purified extracellular streptococcal antigens. Sera with known antibody titers towards these individual components could then be used as controls.

LITERATURE CITED