Extremely High Titers of Serum Antibodies Against the Streptococcal Exoenzyme Deoxyribonuclease B

BENGT HEDERSTEDT,1* STIG E. HOLM,2 AND RENÉE NORBERG3

Department of Bacteriology, National Bacteriological Laboratory, S-105 21 Stockholm,1 Department of Clinical Bacteriology, University of Umeå, S-901 85 Umeå,2 and Department of Immunology, National Bacteriological Laboratory, S-105 21 Stockholm,3 Sweden

In sera from 4 of 25,000 individuals tested for antibodies against streptococci, extremely high antideoxyribonuclease B titers (>10^6 U/ml) were found. Two of the cases were diagnosed as monoclonal gammopathies. The M-components were shown to possess the anti-deoxyribonuclease B activity. The other two cases were diagnosed as relapsing erysipelas. The high serum titers of deoxyribonuclease B antibodies were accompanied by a very high inflammatory reactivity in the patients’ sera and by an oligopolyclonal pattern of immunoglobulin G. In routine diagnoses of streptococcal infections with the anti-deoxyribonuclease B test, patients with extremely high serum titers should be examined for the possible occurrence of gammopathies.

The anti-deoxyribonuclease B (ADNase B) test is said to be the best single test for the serological detection of streptococcal infections (10). The superiority of the ADNase B test to the “classical” anti-streptolysin O (ASO) test for documenting evidence of streptococcal infections of the skin has been well established in recent years (1, 2). The ADNase B test was adopted in our laboratory in 1974. The test has been run in parallel with the ASO test in the routine serological diagnoses of infections by group A streptococci. The diagnoses were considered to be improved not only in infections of the skin but also in β-streptococcal infections in general.

The ADNase B test appears to be highly specific, and, unlike the ASO test, there are no reports so far on specified diseases causing “false-positive” reactions. In the present paper, however, are reported four cases with extremely high ADNase B titers, the specificities of which have been studied and are discussed.

MATERIALS AND METHODS

The sera were obtained from blood samples successively drawn at various intervals from each of four patients at three different hospitals in Sweden. The four patients were selected from around 25,000 individuals whose blood was sent to the laboratory during the years 1974 to 1977 for routine serological testing for antibodies against streptococci (ASO and ADNase B tests). If not otherwise stated, all the serum samples (stored at −70°C) from each patient were tested on the same day.

ASO test. The ASO test was performed by the macromethod of Ibsen (7) as described previously (6). A titer of >200 IU/ml was regarded as elevated (7).

ADNase B test. The ADNase B test was performed by the methyl-green-microtechnique of Nelson et al. (11) as described in an earlier paper (6). A titer of >400 U/ml was regarded as elevated.

AH test. For the anti-streptohyalurinodase (AH) test the reagents of the Bacto-AHT kit (Difco Laboratories, Detroit, Mich.) and the technique recommended by the manufacturer of the kit were used as described previously (6). A titer of >256 U/ml was regarded as elevated (13).

ID test. For the immunodiffusion (ID) test analyses were made by the method described by Wadsworth (15) as reported previously (6).

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

Agarose gel electrophoresis. Agarose gel electrophoresis was performed as described by Johansson (8). For analyzing ADNase B in various fractions, the serum was applied to several gel slits. After the electrophoretic run, one strip was stained by Coomassie brilliant blue and, guided by the pattern obtained, the other strips were cut into pieces. The gel pieces corresponding to the same fractions were pooled, eluted with phosphate-buffered saline, and centrifuged at 10,000 × g for 30 min, and the supernatant was analyzed for ADNase B activity.

Crossed immunoelectrophoresis. Crossed immunoelectrophoresis was done by the method of Ganrot (4) with monospecific rabbit antisera against μ-, α-, γ-, kappa-, and lambda-chains.

RESULTS

Case reports of four patients whose serum titers in the ADNase B test were found to be extremely high (>10^6 U/ml) are presented below.

Case I. This case involved a woman who was born in 1894 and had, since 1945, been nursed almost continuously in a mental hospital for
schizophrenia. The patient had not suffered from any noteworthy infections or other diseases.

In 1969, a high erythrocyte sedimentation rate (ESR) of 122 mm/h was noticed. Serum protein electrophoresis showed a small M-component (0.6 g/100 ml).

In 1975, the M-component in the serum was found to be 1.4 g/100 ml and of the immunoglobulin A (IgA)-lambda class. Small amounts of Bence-Jones protein were detected in the urine. The ASO titer was 50 U/ml, but the ADNase B titer was extremely high (around $5 \times 10^7$ U/ml). This unexpectedly high titer indicated the need for further serological investigations.

ADNase B titers of $>10^6$ U/ml were recorded in five different serum samples collected from 1975 to 1977. The corresponding ASO titers were 50 to 100 IU/ml, and the AH titer was $<32$ U/ml. Serum tested by the ID test gave no precipitation lines against a standardized mixture of streptococcal exoenzymes, suggesting that the patient had not suffered from a recent streptococcal infection. The crossed immunoelectrophoresis of serum against the rabbit anti-a-chain serum is shown in Fig. 1. After electrophoresis, the agarose gel strips were cut into three fractions (Fig. 1). The ADNase B activity was demonstrated in the fraction corresponding to the IgA M-component (fraction 2).

**Case II.** This case involved a 33-year-old man who in August 1976 had an acute myocardial infarction with venous thrombosis of the right lower limb and a pulmonary embolism.

He had previously been healthy, and there was no history of increased disposition to infectious diseases.

The patient recovered, and in January 1977 the ESR was 5 mm. Serum electrophoresis, however, showed a narrow monoclonal component in the $\beta$-region. (Upon recontrol, the M-component was also found in the electrophoresis performed 4 months earlier.) Free, light chains were found in the urine. An X ray of the skeleton revealed no signs of myelomatosis.

In August 1976, the ASO titer was found to be 50 IU/ml, whereas the ADNase B titer was $3.2 \times 10^7$ U/ml. No significant changes in titers were demonstrated in serum samples taken at various intervals during the following 15 months. Extended serological streptococcal examinations revealed an AH titer of $<32$ U/ml and only one precipitation line by the ID test, indicating the monospecificity of the DNase B antibodies.

By crossed immunoelectrophoresis the M-component was shown to be of the IgG-lambda class. The ADNase B activity resided only in the fraction with the M-component (Fig. 2).

**Case III.** This case involved a 66-year-old woman who on 16 November 1974 fell ill, with a fever of 40°C and malaise. After 2 days, erysipelas was diagnosed. Phenoxyethylpenicillin (5.2 g) and cloxacillin (8.0 g) were given orally for 10 days. On 3 December she was febrile again, with a fever of 39.9°C, and a clinical relapse was diagnosed.

Upon admission to the hospital on 15 December, an abundance of $\beta$-streptococci group A and *Staphylococcus aureus* was cultured from the partly ulcerated exanematous area of the left foot. The ESR was 66 mm/h, and the leukocyte count was $10.3 \times 10^9$/liter. Phenoxyethylpenicillin (5.2 g) and cloxacillin (8.0 g) were given daily for 15 days. Table 1 shows the results of the determinations of the ADNase B, ASO, AH, and ID tests of serum samples taken at various dates.

Serum protein electrophoresis of a serum sample collected on 6 December revealed a high inflammatory reaction, with increased values for haptoglobin and orosomucoid. Normal values for C3 and C4 factors were demonstrated. Agarose gel electrophoresis showed a normal pattern, and the ADNase B activity was distributed within the whole gamma globulin fraction.

**Case IV.** This case involved a 49-year-old woman who was admitted to the hospital for a bullous necrotizing erysipelas of the right foot and ankle. She had earlier been treated for varicose veins of the right leg.

Except for epidemic hepatitis in 1950, she had not suffered from any long-lasting infectious diseases in her adult life.

Before the admittance, she had been ill for
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approximately 1 week, with a fever not exceeding 38°C.

Admission laboratory data showed the following: an ESR of 127 mm/h; a leukocyte count of 17.9 × 10⁹/liter (93% neutrophils); slightly increased serum values for aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase; and microscopic hematuria. Serum electrophoresis showed a very high inflammatory response and oligoclonal hypergammaglobulinemia. The results of the streptococcal antibody analyses are shown in Table 1. The ADNase B titer was very high, whereas the ASO and AH titers were found to be just over the upper limits of normal. Four precipitation lines were recorded by the ID test. The ADNase B activity was distributed over the entire electrophoretic gamma globulin fraction.

The patient was given phenoxymethylpenicillin (2.6 g) and cloxacillin (8.0 g) daily for 34 days. After 13 days of this treatment, there was a clinical relapse, and β-streptococcal group A were cultured from ulcers of the leg. The same antibiotic treatment as before was given for 1 month, with good results, and the patient was subjected to skin transplantation.

DISCUSSION

Based on the nature of their diseases, the present four patients with extremely high serum titers of DNase B antibodies could be separated into two groups. Cases I and II were diagnosed as monoclonal gammopathies, and cases III and IV were diagnosed as relapsing erysipelas.

In the first two cases, the M-proteins (of the IgA class in case I and the IgG class in case II) possessed the ADNase B activity. M-components with antibody activity against various antigens, among them streptolysin O, have been reported during the last decades (14). These findings have suggested that at least some monoclonal gammopathies may develop as the result of antigenic stimulation in genetically susceptible individuals (16). Monoclonal gammopathies have been experimentally induced in rabbits by prolonged stimulation with streptococcal antigens (12), and recently, Kalliomäki et al. (9) described a patient with a strikingly long history of streptococcal infections that finally might have caused the development of M-components with ASO activity.

DNase B antibodies are probably as common as antibodies against streptolysin O in streptococcal diseases. Though not previously reported, it might not be surprising that ADNase B activity also occasionally occurs in M-components of patients with monoclonal gammopathies.

Neither of the present two patients with M-components had apparently suffered from long-lasting infectious diseases. On the other hand, streptococcal antigens are very common antigens, and even repeated subclinical streptococcal infections might give rise to malignant transformation in the plasma cell line.

The two patients with relapsing erysipelas had developed very high titers of DNase B antibodies.

### Table 1. Results of examination with the ADNase B, ASO, AH, and ID tests of serum samples taken at various dates from patients with relapsing erysipelas

<table>
<thead>
<tr>
<th>Case</th>
<th>Date</th>
<th>ADNase B</th>
<th>ASO</th>
<th>AH</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>18 November</td>
<td>400</td>
<td>140</td>
<td>512</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>6 December</td>
<td>1.6 × 10⁶</td>
<td>100</td>
<td>1,024</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>30 December</td>
<td>6,400</td>
<td>100</td>
<td>2,048</td>
<td>ND</td>
</tr>
<tr>
<td>IV</td>
<td>27 January</td>
<td>2.4 × 10⁶</td>
<td>400</td>
<td>1,024</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>6 February</td>
<td>4.8 × 10⁶</td>
<td>560</td>
<td>1,024</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>24 February</td>
<td>25,600</td>
<td>400</td>
<td>1,024</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>20 March</td>
<td>12,800</td>
<td>400</td>
<td>1,024</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Case III, 1974; the patient was treated with antibiotics 16 to 25 November and 6 to 22 December. Case IV, 1975; the patient was treated with antibiotics 24 January to 26 February and 11 March to 10 April.

* Results for ADNase B, ASO, and AH tests are given in units per milliliter. Results for the ID test are given as the number of precipitation lines.

* ND, Not determined.
ies of the IgG class. These high titers were accompanied by a very high inflammatory reactivity in the patients’ sera and an oligopolyclonal pattern of IgG.

A remarkable feature in both of the erysipelas cases was the rapid decrease of the ADNase B titer. Within 23 days, which is the half-life of IgG (3), there appeared to be an antibody decay of much more than 50%. One possible explanation would be that the titer decrease was a result of an immune complex formation. However, other than a transient hematuria in one of the patients, none of the patients showed any signs of immune complex diseases which would have favored that hypothesis.

The reports of the present four patients might lead to the following conclusion. In routine serological diagnoses of streptococcal infections with the ADNase B test, extremely high titers will be found in rare cases. Particularly if there are no signs of current streptococcal infection in those cases, the patient should be examined for possible occurrence of gammopathies.

The present findings are not contradictory to the common concept that the ADNase B test shows a very high degree of specificity.

LITERATURE CITED