Study on Serum Antistreptococcal Esterase among School Children

TSUNE TAKEUCHI, M.D., JUNZO INOUE, M.D.
TOYOHIKO ONISHI, M.D., AND SEIICHI KAWAKITA, M.D.*

Antistreptococcal esterase (an antibody to extracellular esterase produced by Group A streptococci: ASE) was evaluated according to Hayano’s method by using an assay kit on sera of children from two schools, one (F-school) in a non-epidemic state and the other (I-school) in an epidemic state of Group A streptococcal infection.

Out of 104 children of F-school, 51 with negative throat cultures for Group A streptococci showed a mean serum ASE titer of 191 ± 144 unit, and 53 with positive throat cultures for Group A streptococci showed a mean serum ASE titer of 360 ± 147 unit.

Follow-up determinations on serum antibodies to four Group A streptococcal antigens were performed on the children from I-school during a period of 13 months after an epidemic infection of Group A streptococci. The initially elevated serum ASE titer showed a decreasing trend on subsequent bleedings during the study period as well as antistreptolysin O (ASO) and antideoxyribonuclease B (ADN-B) titers. However, the changing pattern of the antistreptococcal polysaccharide (ASP) titer was different from that of the other three antibodies. A positive correlation was observed statistically between ASE and ASO titers, and also between ASE and ADN-B titers. The ASE tests appear to be useful for serological evidence of a streptococcal infection.

Hayano and Tanaka1,2 have reported a method for the quantitative estimation of antibodies to extracellular esterase produced by Group A and B streptococci, and applied to the determination of an ASE titer on the sera of patients with scarlet fever.

In the present studies, ASE titer was measured according to Hayano’s method by using an assay kit supplied from the Dainippon Pharmaceutical Co. Ltd. on sera of children from two schools, that is, one in a non-epidemic state and the other in an epidemic or postepidemic state of Group A streptococcal infection. The results of the ASE titer were compared with antibody titers of other streptococcal antigens.

MATERIALS AND METHODS

In 1982, throat cultures for beta-hemolytic streptococci were performed on 674 children of a primary school (F-school) in Otsu city. Epidemic infections of Group A streptococci were not observed among the children of this school at the time of study. These children were divided into two groups depending upon the isolation of Group A hemolytic streptococci during the study period. The sera of each group were analyzed simultaneously for two different streptococcal antibodies.

In 1980, epidemic infections of Group A streptococci occurred among the children of a school (I-school) in Kyoto city during August.

Key Words:
Group A streptococcal infection Antistreptococcal esterase (ASE) Antistreptolysin O (ASO) Antideoxyribonuclease B (ADN-B) Antistreptococcal polysaccharide (ASP)

Otsu Medical Association and *Shiga University of Medical Science, Otsu, Japan
Mailing address: Seiichi Kawakita, M.D., The First Department of Internal Medicine, Shiga University of Medical Science, Seta, Otsu 520-21, Japan

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### TABLE I VARIATION OF TITERS OF ANTI-STREPTOCOCCAL ANTIBODIES IN FOLLOW-UP STUDY

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<tr>
<td>ASE</td>
<td>( \bar{X} )</td>
<td>327</td>
<td>269</td>
<td>268</td>
<td>225</td>
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<tr>
<td></td>
<td>SD</td>
<td>166</td>
<td>163</td>
<td>160</td>
<td>149</td>
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<tr>
<td>ASO</td>
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<td>457</td>
<td>369</td>
<td>310</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>389</td>
<td>334</td>
<td>251</td>
<td>147</td>
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<tr>
<td>ADN-B</td>
<td>( \bar{X} )</td>
<td>964</td>
<td>877</td>
<td>668</td>
<td>645</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>500</td>
<td>448</td>
<td>474</td>
<td>430</td>
</tr>
<tr>
<td>ASP</td>
<td>( \bar{X} )</td>
<td>104</td>
<td>115</td>
<td>111</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>85</td>
<td>88</td>
<td>76</td>
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<tr>
<td>n</td>
<td>23</td>
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and October. Follow-up studies for carrier rates of Group A streptococci in their throats and the serial determinations of their serum antibodies to three antigens of Group A streptococci (ASO, ADN-B, and ASP) were performed among these children during October of 1980 and November of 1981. The results of the studies have been reported in a previous paper. In the present study the determination of ASE titers on 121 sera which had been obtained from these children was performed, and the results were compared with ASO, ADN-B and ASP titers evaluated on the same sera.

Throat cultures and isolation of beta-hemolytic streptococci were performed as previously described.

Determination of the ASE titer: The ASE titer was determined by using an ASE Kit. Two types of extracellular esterase produced by Group A streptococci (STE A) were classified as STE A-1 and STE A-2. STE A-1 was used as an antigen in the ASE kit. This test was performed by adding 0.1 ml of esterase and 0.1 ml of staphylococcal protein A attached to a cell wall to 0.1 ml of a 1:100 dilution of serum with distilled water. After incubation for 30 minutes at 37°C and then centrifugation, 1 ml of DTNB (2-nitro-5-thiobenzoic acid) and 0.1 ml of s-acetyl thiophenol were added to the separated antigen-antibody complex. After incubation for 30 minutes at 37°C, an assay of the ASE was performed by reading the optical density of the supernatant of this complex.

Determination of the ASO titer: The ASO titer was determined according to Rantz and Randall's method.

Determination of the ADN-B titer: The ADN-B titer was measured by using a Streptonase-B Kit (Wampole Laboratories).

Determination of the ASP titer: The ASP titer was measured according to the hemagglutination method by using sheep erythrocytes which were sensitized with polysaccharide extracted from Group A streptococcal cell walls.

### RESULTS

I. Studies on the sera of children from F-school:

Group A streptococci were isolated from 91 (13.5%) out of the 674 children. Sera taken from 51 children with negative throat cultures for Group A streptococci and 53 children out of the 91 with positive throat cultures for Group A streptococci were tested for ASE and ASO titers.

1) Determination of ASE titer:

In 51 children with negative throat cultures for Group A streptococci, the mean serum ASE titer was 191 ± 144 units. Out of these 51 children, 9 (17.6%) had an elevated ASE titer of 350 units or more. In 53 children with positive throat cultures for Group A streptococci, the mean serum ASE titer was 360 ± 147 units. Out of the 53 children, 30 (56.6%) had an elevated ASE titer of 350 units or more.

2) Correlation between the serum ASE and ASO titers:

The results of the ASE titer were compared with the results of the ASO titer in a total number of 104 serum samples. We found a positive correlation between the serum ASE and ASO titers (r = 0.6303).

II. Studies on the sera of children from I-school:

1) The variation of titers of serum anti-

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Relationship between ASE and ASO titers.

\[ n = 121 \quad \gamma = 0.6180 \]

Fig. 1.

Relationship between ASE and ADNase-B titers.

\[ n = 121 \quad \gamma = 0.5737 \]

Fig. 2.

streptococcal antibodies in the follow-up study:

A total number of serum samples tested in the follow-up study was 121 specimens. The results of the serum antibody titers to the four antigens of Group A streptococci are presented in Table I. Initially elevated ASE titer showed a decreasing trend as well as elevated ASO and ADN-B titers during the study period. In contrast, the changing pattern of the ASP titer was different from the three antibody responses, and the peak level of the ASP titer was reached after two months.

2) Correlation between the serum ASE and ASO titers:

The relation between the serum ASE and ASO titers is shown in Fig. 1. Out of the 121 sera, twenty-nine samples (24.0%) had an elevated ASE titer of 350 units or more and also an elevated ASO titer of 333 units or more. Ten samples (8.3%) had an elevated ASE titer of 350 units or more but a normal ASO titer below 250 units. Thirty-eight samples (31.4%) had a normal ASE titer below 350 units but an elevated ASO titer of 333 units or more. When the results of the ASE titer were compared with those of the ASO titer in total number of 121 samples, we found a positive correlation between the serum ASE and ASO titers ($r = 0.6180, \gamma = 0.0017x + 2.0098$).

3) Correlation between ASE and ADN-B titers:

The relation between the ASE and ADN-B titers are presented in Fig. 2. Out of 121 sera, thirty-three samples (27.3%) had an elevated ASE titer of 350 units or more and also an elevated ADN-B titer of 680 units or more. Nine samples (7.4%) had an elevated ASE titer of 350 units or more but a normal ADN-B titer below 480 units. Thirty-four samples (28.1%) had a normal ASE titer below 350 units but an elevated ADN-B titer of 680 units or more. When the results of ASE titer were compared with those of the ADN-B titer in a total number of 121
samples, we find a positive correlation between the serum ASE and ADN-B titer (r = 0.5737, y = 0.0017x + 2.2263).

4) Correlation between ASE and ASP titer:

The relation between ASE and ASP titers is presented in Fig. 3. Out of the 121 sera, 11 samples (9.0%) had an elevated ASE titer of 350 units or more and also an elevated ASP titer of 256 units or more. Eleven samples (9.0%) had an elevated ASE titer of 350 units or more but a normal ASP titer below 128 units. Twelve samples (9.9%) had a normal ASE titer below 350 units but an elevated ASP titer of 256 units or more. When we compare the results of the ASE titer with those of the ASP titer in a total number of 121 serum samples, we could not find a positive correlation between the ASE and ASP titers (r = 0.09032, y = 0.00027x + 1.80197).

DISCUSSION

The ASO test is the most widely used serological test for the detection of Group A streptococcal infections. Ayoub and Wannamaker have asserted that the ADN-B and antinicotinamide adenine dinucleotidase tests will show an elevation of at least one antibody in approximately 95% of rheumatic fever cases. Thus, it appears that using a combination of antibody tests will increase the percentage of evidence for Group A streptococcal infections. Recently, Hayano has discovered the usefulness of the ASE test to detect the streptococcal infection. In this report the results of the ASE titer are compared with those of the ASO, ADN-B and ASP titer on sera taken from children.

The mean serum ASE titer of 53 children with positive throat cultures for Group A streptococci and 51 children with negative throat cultures for Group A streptococci in F-school were 360 ± 147 and 191 ± 144 units, respectively. An serum ASE titer of 350 units or more was observed in 56.6% of the children with positive throat cultures for Group A streptococci, and in 17.6% of children with negative throat cultures for Group A streptococci. This data indicates that the serum ASE titer shows such a rise in about half of the cases of carriers of Group A streptococci among school children.

In the follow-up study on the serum antibody titers to four antigens of Group A streptococci after the epidemic streptococcal infection in I-school, initially elevated ASE titer showed a similar decreasing trend as well as ASO and ADN-ADN-B titers during the convalescent period. Moreover, we found a positive correlation between the ASE and ASO titers, and between the ASE and ADN-B titers. However, discordance was observed between the ASE and ASO titers in 39.7% of the 121 cases, and between the ASE and ADN-B titers in 35.5% of the 121 cases. A positive correlation between the ASE and ASP titers was not observed.

Hayano has reported that variation of the serum ASE titer showed a similar tendency to the variation of the ASO titer after streptococcal infection. The data of the present study seem to show that the simultaneous determination of the serum ASE titer together with the ASO and ADN-B titers would assist in the diagnosis of Group A streptococcal infection.

REFERENCES