Infection of Group A Streptococcus and Antibody Response to Extracellular Antigens

SEIICHI KAWAKITA, M.D., TSUNE TAKEUCHI, M.D*, JUNZO INOUE, M.D*
TOYOHICO ONISHI, M.D*, AND YOSHIO UEMURA, M.D*

A study demonstrating the relationship between pharyngeal infection of group A streptococci and the antibody response was performed. Group A streptococci were recovered from 44 (9.4%) of 466 children, of which 41 strains were typable by T-protein. Fifty-five percent of 41 children from whom group A streptococci were recovered showed a significant rise in antistreptolysin O (ASO) titers in sera. In children from whom group A streptococci were recovered and with elevated ASO titers, titers of antideoxyribonuclease B (ADNase B) were significantly higher than those who were negative for group A streptococci and also showed elevated or normal titers for ASO. Patients with acute rheumatic fever demonstrated elevated titers of both ASO and ADNase B. These findings suggest that a combination of ASO and ADNase B titers should be applied to diagnose the streptococcal infections. Antinicotinamideadenine dinucleotidase showed a low level of titers in children who had group A streptococci in their throat and exhibited elevated titers in streptococcal antibodies, and also in patients with acute rheumatic fever.

IT has been established that acute rheumatic fever and rheumatic heart disease occur following antecedent group A streptococcal infections in upper respiratory tract. Throat cultures carefully done are necessary for determining streptococcal infections. At the same time, in patients with acute rheumatic fever streptococcal antibody tests are in general a more reliable indicator of recent streptococcal infections than throat cultures. The present investigation was done to provide informations on the

value of streptococcal antibody in both streptococcal infections and rheumatic fever.

MATERIALS AND METHODS

The epidemiological study of streptococcal infections was done in apparently healthy children 6 to 11 years of age in one school of Otsu city. Three patients with typical acute rheumatic fever were enrolled in this study.

1. Throat cultures were collected on swabs from 466 elementary school children during the period from October to November 1978, Otsu city, Shiga prefecture. Isolated β -hemolytic streptococci were serologically grouped and all strains of group A were typed according to the T-protein agglutination technique by use of anti-T protein specific sera.

2. Blood samples were employed in the determination of 3 antibodies to streptococcal

Key Words:

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Antihyaluronidase (AH)

The First Department of Internal Medicine, School of Medicine, Shiga University of Medical Science, Otsu 520-21, Japan

**The Committee of Streptococcal Disease, Shiga Medical Association, Otsu, Japan*

TABLE I NUMBER AND PERCENTAGE OF BETA-HEMOLYTIC STREPTOCOCCI ISOLATED FROM THE CHILDREN OF ELEMENTARY SCHOOL IN OTSU CITY IN 1978 (FUJIO SCHOOL)

No. of culture taken	466
No. of strains isolated	111
Prevalence (%)	23.8
Serological groups	
Group A	44 (9.4%)
B	17 (3.6%)
C	5 (1.0%)
G	30 (6.4%)
ungroupable	15 (3.2%)

extracellular products.

1) Antistreptolysin O (ASO) titer was determined according to Rantz and Randall's method¹

2) Antideoxyribonuclease B (ADNase B) titer was assayed according to a modification of microtechnique method of Nelson² Streptococcal deoxyribonuclease B (DNase B) was supplied from Behring Institute. All sera were inactivated by heating. Initial dilutions of 1:50 and 1:75 were made with imidazol buffer and further 2 fold dilutions were carried out. A range of dilution from 1:50 to 1:4,800 was provided. Amounts of 0.025 ml DNase B antigen (30 U/ml) were added to diluted sera in microplates. The plates were tapped gently, but thoroughly, to mix, and then DNA-Toluidin blue substrate was added to this mixture. After incubation at 37°C for 4 hr, the DNA-Toluidin blue substrate complex showed blue precipitate when intact, and lost precipitation in relative proportion to its digestion by DNase, so that complete digestion resulted in almost total loss of precipitate. The titer on the serum assay was read

as the reciprocal of the serum dilution which showed definite inhibition of enzyme activity.³

3) Antinicotinamideadenine dinucleotidase (ANADase) titer was measured according to a modification of reduction by alcohol dehydrogenase by Petersen's method⁴ Streptococcal NADase was supplied from Behring Institute. Serum dilution spanning the range from 1:20 through 1:240 was made. To each serum dilution, nicotinamideadenine dinucleotidase (NADase) solution was pipetted in small tubes. Tubes were left to stand in a waterbath at 37°C for 30 min. After that NAD solution was pipetted into each tube at 30 sec and incubated at 37°C for a further 7.5 min. Then alcohol dehydrogenase suspension, ethanol and semicarbazide were added to each preparation. The extinction of the individual serial dilutions was measured at 366 nm in photometer. The positive serum dilution was that which gave an extinction value nearest to 0.250⁵

4) Antihyaluronidase (AH) test was performed according to the mucin clot prevention technique by using a commercially available reagent⁶

RESULTS

The Prevalence of β -Hemolytic Streptococci in School Children

β -hemolytic streptococci were isolated from 111 of the 466 children and the recovery rate was 23.8%. Forty-four of the 111 was group A strains. Thus the frequency of recovery of group A was 9.4%. The group of β -hemolytic streptococci other than group A most often identified in the children were group G (6.4%), group B (3.6%) and group C (1.0%) (Table I). Of the 44 group A strains isolated, 41 was typable by T-protein, the common of which was types 1, 12, B-3264 and 4 (Table II).

TABLE II DISTRIBUTION OF T-TYPE OF GROUP A STREPTOCOCCI ISOLATED FROM THE CHILDREN OF ELEMENTARY SCHOOL IN OTSU CITY, IN 1978. (FUJIO SCHOOL)

Serological T-type	No. of strains	Percentage (%)
1	15	3.2
4	7	1.5
12	10	2.1
B-3264	9	1.9
untypable	3	0.6
Total	44

Fig.1. Comparison of ASO titer in children of elementary school with positive and negative throat culture for group A streptococci.

The Determination of Antibody

1) Serum ASO titers were determined for the 404 children (Fig. 1). It is generally accepted that the upper limits of normal titer for ASO in school age children was 333 U/ml. Significant rise in titers of ASO was found in 55.8% of 43 children with positive cultures for group A streptococci. In contrast, only 18.3% of 361 children with negative cultures for group A streptococci showed a significant rise in titers of ASO.

2) Eighty-four sera of 404 children were analyzed for titers of both ADNase B and ANADase. For establishment of upper limits of normal values of ADNase B and ANADase and exploration of the meaning of increased antibody, 84 children were grouped in 3 categories (Figs. 2 and 3).

- (1) Group 1: Twelve children positive for group A streptococci in their throats and with a significant ASO response.
- (2) Group 2: Twelve children negative for group A streptococci and with a significant ASO response.
- (3) Group 3: Sixty children negative for group

A streptococci and with normal titers of ASO.

The geometric mean titers (GMTs) for 3 antibodies in Group 1 were 939U for the ASO, 765 for the ADNase B and 86U for the ANADase. All of sera showed the titers of 300U or higher for the ADNase B. Eleven of 12 sera (91.6%) showed titers of 50U or more for the ANADase.

The GMTs for the 3 antibodies in Group 2 were 600U for the ASO, 256U for the ADNase B and 51U for the ANADase. Eight of 12 sera (66.6%) demonstrated the titers of 300U or higher for the ADNase B and 5 of 12 sera (41.7%) had the titers of 50U or more for the ANADase.

The GMTs for the 3 antibodies in Group 3 were 77U for the ASO, 54U for the ADNase B and 16U for the ANADase. There was one serum which showed the titer of 300U for the ADNase B and only one serum showed the titer of 50U for the ANADase.

Three patients with acute rheumatic fever showed a significant elevation of titers for the ADNase B (GMTs: 2306) in comparison with the other groups. However, the titers of ANADase (GMTs: 79) did not show the striking

Fig.2. Distribution of antideoxyribonuclease B (anti-DNase B) titer in children of elementary school with positive and negative throat culture for group A streptococci, and in patient with acute rheumatic fever.

difference between rheumatic fever and the other groups.

Follow Up Study of Antibody Response of Rheumatic Fever

Sera from acute and convalescent phases were available from 3 patients with acute rheumatic fever and rheumatic heart disease, and analyzed for the titers of ASO, ADNase B, ANADase, and AH.

1) Twenty-two year-old female admitted on 14, April, 1973 to the hospital with history of sore throat, migratory polyarthritis and dyspnea. She had a previous history of rheumatic fever at age 16. Physical findings included cardiomegaly and apical pansystolic murmur. Following the treatment with combination of oral prednisolone, penicillin and digitalis, laboratory findings gradually improved. The levels of streptococcal antibodies declined with considerable regularity after the treatment and reached to the normal

levels after one year from the initial attack (Fig. 4).

2) Forty-four year-old female admitted on 3, July, 1973 to the hospital with history of sore throat, migratory polyarthritis, and dyspnea. She had a previous history of rheumatic fever at age 28. Physical findings included cardiomegaly, basal early diastolic murmur, and heart rate of 100 per min. Following the treatment with combination of oral prednisolone (60 mg/day) and cephalotin, titers of ASO, AH and ADNase B showed rapidly decreased tendency and reached to the normal value after 3 months. However, titers of ANADase showed a low level on the initial sera and no changes were found on the convalescent sera.

3) Sixteen year-old female admitted on 25, June, 1977 to the hospital with a history of sore throat, migratory polyarthritis. Physical findings included basal early diastolic murmur, and tachycardia (120/min). Following the treatment with

Fig.3. Distribution of antinicotinamideadenine-dinucleotidase (anti-NADase), in children of elementary school with positive and negative throat culture for group A streptococci, and in patient of acute rheumatic fever.

Fig.4. Immunologic response in acute rheumatic fever. M.Y. 22y.

Fig 5. Immunologic response in acute rheumatic fever. Y.Y. 16y.

combination of oral prednisolone and erythromycin, laboratory findings gradually improved, and ASO titers reached to the normal value after 4 months. However, ADNase B titers remained elevated over the 17-week period, and ANADase showed low titers in the initial and convalescent stages (Fig. 5).

DISCUSSION

The data obtained from this study showed the relationship between pharyngeal infection of group A streptococci and the response of streptococcal antibodies in a closed population. It has been insisted by Kaplan⁷ that the differentiation of the active infection from streptococcal carrier depended on the recovery of the organism plus a subsequent rise in titer of antibody. According to Ayoub's study⁸ a high titer for the ADNase B was observed in patients with acute rheumatic fever while a definite high titer for the ANADase was seen in patients with acute glomerulonephritis. Moreover, Kaplan⁹ described that there were the seasonal fluctuations in titers for 3 antibodies during the 2-year period, and the ADNase B titers tended to be higher than the ASO and ANADase titers.

Our study confirms that elementary school children harbored different type of group A streptococci which were detected in the frequency of 9.4% of all children. Of the 43 children who harbored group A streptococci in their throat, 55.8% showed a rise in ASO titers at the initial state. However, of the 361 children from

whom group A streptococci were not recovered from their throat, only 18.3% showed a rise in ASO titers. ADNase B titers were significantly higher in the children who harbored group A streptococci and with elevated titers of ASO than the children who did not harbor group A streptococci and with the normal levels of ASO titers. On the contrary, the ANADase titers were lower than those of the other 2 antibodies. Three patients with acute rheumatic fever showed significantly elevated titers for both ASO and ADNase B in contrast to low levels of ANADase titers. Follow-up study of ASO and ADNase B titers for patients with rheumatic fever over a 21-week period showed a gradual decline from the 6th week after the initial attack.¹⁰ Our study showed a similar tendency of a decline of antibody titers. Recent study by Tiesler¹¹ demonstrated that a combination of the determination of ADNase B, ANADase and AH antibodies was best for the diagnosis of group A streptococcal upper respiratory infections. Moreover, Kaplan indicated that ADNase B is the antibody of choice in studying streptococcal infections of both the upper respiratory tract and the skin.¹²

From the above mentioned study, a combination of ASO and ADNase B tests is considered to be useful for the diagnosis of recent upper respiratory infections of group A streptococci.

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