Study of Antibody Response to 4 Streptococcal Antigens in Rheumatic Fever and Kawasaki Disease with or without Cardiovascular Lesions

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Patients with rheumatic fever (RF) and Kawasaki disease (MCLS) were divided into two groups, one with and the other without cardiovascular lesions, and their sera were examined for antibodies to 4 streptococcal antigens: anti-streptococcal polysaccharide (ASP), anti-deoxyribonuclease B (ADN-B), anti-streptolysin O (ASO) and anti-streptokinase (ASK) in order to clarify the relationship between group A streptococci and these two diseases.

In RF the frequency of positive levels of ASP at its early stage was lower than those of the other 3 antibodies, but the ASP titer tended to persist at elevated levels. The frequency of ADN-B in patients with rheumatic heart disease (RHD) was higher than that in those without RHD, but with regard to the other antibodies no difference was found between the two. Of the 4 antibodies the frequency of at least one positive level was 100% in all the sera of RF patients within 3 months from onset.

The differences in the frequencies of positive levels of all 4 antibodies in both MCLS and the controls did not prove to be significant. The frequencies of ASP, ADN-B and ASO in the sera of patients without cardiovascular lesions tended to be slightly higher than those in patients with cardiovascular lesions.

RHEUMATIC fever (RF) is an inflammatory disease that is usually a sequela to an infection caused by group A streptococci and induces carditis in more than 50% of all patients.¹

Kawasaki disease (MCLS) resembles acute streptococcosis in its clinical symptoms and also brings about cardiovascular lesions in 20 to 40% of all patients.

The aim of the present study was to observe in RF and MCLS with or without cardiovascular lesions the differences in antibody responses to 4

streptococcal antigens, that is anti-streptococcal polysaccharide (ASP) as an antibody to a cellular component and anti-deoxyribonuclease B (ADN-B), anti-streptolysin O (ASO) and anti-streptokinase (ASK) as antibodies to extracellular substances.

MATERIALS AND METHODS

As controls, the ASP titers of 90 sera and those of ADN-B of 71 sera from patients without infectious diseases and immunological disorders were determined and divided according to their respective age group, the first being that of patients less than 6 years of age and the other that of patients aged from 6 to 15 years.

Sixty-seven sera from 28 patients with RF and

Key words:

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TABLE I THE NORMAL RANGE OF TITERS IN ANTIBODIES TO 4 STREPTOCOCCAL ANTIGENS FOR CHILDREN LESS THAN 6 YEARS AND FROM 6 YEARS TO 15 YEARS OF AGE

<i>Antibodies</i>	<i>Ages</i>	<i>Titers of upper limits</i>	<i>Titers of positive levels</i>
A S P	0 – 5 years	16	32 ≤
	6 – 15 years	32	64 ≤
ADN-B	0 – 5 years	60	85 ≤
	6 – 15 years	240	340 ≤
A S O	0 – 5 years	40	60 ≤
	6 – 15 years	240	320 ≤
A S K	0 – 5 years	640	1280 ≤
	6 – 15 years	1280	2560 ≤

TABLE II THE FREQUENCIES OF POSITIVE LEVELS OF 4 STREPTOCOCCAL ANTIBODIES IN THE SERA OF PATIENTS WITH RHEUMATIC FEVER (RF) AT TWO DIFFERENT STAGES FROM ONSET

<i>Antibodies</i>	<i>Less than 3 months from onset</i>	<i>3 or more than 3 months from onset</i>	<i>Total of sera</i>
A S P	10/20 (50.0%)	9/21 (42.9%)	19/41 (46.3%)
ADN-B	17/20 (85.0%)	8/21 (38.1%)	25/41 (61.0%)
A S O	21/30 (70.0%)	3/37 (8.1%)	24/67 (35.8%)
A S K	24/30 (80.0%)	12/37 (32.4%)	36/67 (53.7%)

TABLE III THE FREQUENCIES OF POSITIVE LEVELS OF 4 STREPTOCOCCAL ANTIBODIES IN THE SERA OF PATIENTS WITH RHEUMATIC FEVER (RF) WITH OR WITHOUT RHEUMATIC HEART DISEASE (RHD)

<i>Antibodies</i>	<i>Without RHD (20)</i>	<i>With RHD (8)</i>	<i>Total (28 cases)</i>
A S P	12 (60.0%)	5 (62.5%)	17 (60.7%)
ADN-B	10 (50.0%)	6 (75.0%)	16 (57.1%)
A S O	12 (60.0%)	5 (62.5%)	17 (60.7%)
A S K	14 (70.0%)	5 (62.5%)	19 (67.9%)

87 sera from 87 patients with MCLS were picked at random for their antibody levels at the clinic of pediatrics of Kagoshima University Hospital. All the patients with RF were either 6 or more than 6 years of age, but the 77 patients with MCLS were less than 6 years of age and the other 10 patients ranged in age from 6 to 15 years.

As to the serological reactions, the ASP titers were determined by means of the modified hemmagglutination method² (ASP kit, Hoekist Japan), the ADN-B titers by the enzyme inhibition method (Hemoprobe B test, imported by Kyorin Seiyaku from Carter Wallance Inst., USA), the ASO titers by the modified method of Rantz and Randall (ASLO-2, Eiken), and the

ASK titers by the kinase test (Serodia ASK, Fujirebio Inc.)

RESULTS

1. Normal antibody value

The upper limits of the normal were defined as those the antibody titers or levels exceeded by no more than 20% of the control children. The upper limits of ASP were found by age to be 16 units of titer for children less than 6 years and 32 units for children 6 to 15 years of age, and for ADN-B they were 60 units of titer for children aged less than 6 years and 240 units for those 6 to 15 years of age.

TABLE IV THE FREQUENCIES OF POSITIVE LEVELS OF 4 STREPTOCOCCAL ANTIBODIES IN THE SERA OF PATIENTS WITH KAWASAKI DISEASE (MCLS) AT TWO DIFFERENT STAGES FROM ONSET

<i>Antibodies</i>	<i>Less than 3 months from onset</i>	<i>3 or more than 3 months from onset</i>	<i>Total of sera</i>
A S P	12/60 (20.0%)	6/27 (22.2%)	18/87 (20.7%)
ADN-B	8/60 (13.3%)	5/27 (18.5%)	13/87 (14.9%)
A S O	6/60 (10.0%)	5/27 (18.5%)	11/87 (12.6%)
A S K	3/60 (5.0%)	2/27 (7.4%)	5/87 (5.7%)

TABLE V THE FREQUENCIES OF POSITIVE LEVELS OF 4 STREPTOCOCCAL ANTIBODIES IN THE SERA OF PATIENTS WITH KAWASAKI DISEASE (MCLS) WITH OR WITHOUT CARDIOVASCULAR LESIONS (CL)

<i>Antibodies</i>	<i>Without CL (39)</i>	<i>With CL (48)</i>	<i>Total (87 cases)</i>
A S P	11 (28.2%)	7 (14.6%)	18 (20.7%)
ADN-B	8 (20.5%)	5 (10.4%)	13 (14.9%)
A S O	7 (17.9%)	4 (8.3%)	11 (12.6%)
A S K	1 (2.6%)	4 (8.3%)	5 (5.7%)

The normal ranges of ASO and ASK were derived from Yamada³ and Suzuki⁴ (Table I)

2. Rheumatic fever (RF)

The frequency of positive levels of ASP was lower than those of the other 3 antibodies within 3 months from onset of disease but higher beyond that period. The frequencies of positive levels of ADN-B, ASO and ASK titers within 3 months from onset were significantly higher than those beyond that period. (ADN-B: $p < 0.006$, ASO: $p < 0.0005$, ASK: $p < 0.0005$) (Table II)

In all 4 antibodies the frequencies of one or more positive antibodies within 3 months from onset were 100% in 20 patients.

When comparing the frequencies of positive levels in patients with rheumatic heart disease (RHD) with those in patients without RHD, no difference was found in any of the frequencies of ASP, ASO and ASK in both groups; in ADN-B with RHD the levels tended to be higher than those in ADN-B without RHD, but the difference was not significant. (Table III)

3. Kawasaki disease (MCLS)

No difference was found in any of the 4 antibodies between the frequencies of positive levels within 3 months from onset on the one hand, and in those beyond 3 months on the other. The frequency of positive levels in ASP tended to be

higher than in the other 3 antibodies. However, the frequencies of ASP and the other 3 antibodies in MCLS did not exceed that of the control group. (Table IV)

When comparing the frequencies of positive levels in patients with cardiovascular lesions (CL) observed by echocardiography with those in patients without CL, the frequencies of ASP, ADN-B and ASO in patients with CL tended to be lower than in patients without CL, though the difference was not significant. (Table V)

DISCUSSION

As rheumatic fever is thought to be a sequela to group A streptococcal infection, various antibodies to streptococcal antigens have been examined.

In the present study it is clear that ASP levels did not rise at an early stage but rather tended to remain at elevated levels later after onset when reaching an inactive stage.

Goldstein et al.⁵ reported that there was a pronounced immunological cross-reaction between the polysaccharide specific to group A streptococci and the structural glycoprotein isolated from heart valves. The difference of ASP levels in patients with RHD and in those without RHD was not found significant, although Dudding et al.⁶ showed that elevated

antibody levels of group A carbohydrate in patients with rheumatic valvulitis were found to persist for periods of at least 1 year and up to 20 years after the last acute attack, while those in patients with chorea or glomerulonephritis declined to within the normal range during the acute illness.

The frequency of positive levels of ADN-B was higher than those of the other 3 antibodies within 3 months from onset, while the ADN-B levels declined to normal later than the others. The ADN-B levels of sera in patients with RHD were higher than in those without RHD.

ASO and ASK levels declined to normal at an earlier stage than did ASP and ADN-B.

As the frequency of at least one positive level of the 4 antibodies was 100% in all the sera of RF patients within 3 months from onset, the batteries of all 4 antistreptococcal antibody titrations were useful for serologic diagnosis of group A streptococcal infection.

MCLS is similar to acute streptococcosis in its clinical symptoms, but its pathogenesis is not clear. In the present study, it was found that the frequencies of positive levels of the 4 antibodies did not change at either of the two stages and that there was no significant difference between MCLS and the controls. The frequencies of ASP,

ADN-B and ASO in the sera of patients without CL were higher than those in the sera of the patients with CL. In conclusion, it can be said that there was no evidence to support the assumption that streptococcal infection is involved in the pathogenesis of MCLS and cardiovascular lesions.

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