Hemagglutination of Formalin Preserved Erythrocytes Treated with Tannic Acid and Sensitized with Streptokinase-Streptodornase (Varidase)

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SUMMARY

Hemagglutination of formalin preserved human group O erythrocytes treated with tannic acid and sensitized with streptokinase-streptodornase (Varidase) were devised by us and investigated in 183 sera of patients suffering from rheumatic fever, acute glomerulonephritis, scarlet fever and chronic tonsillitis etc. High titers were often observed in these poststreptococcal sequelae and titers were commonly low in apparently healthy persons. The titers of the hemagglutination (anti-SK-SD) correlated with that of Todd units of antistreptolysin O, but usually higher than the latter.

It may be useful as one of the serological reactions for the supporting evidence of preceding streptococcal infection in guidance for the diagnosis of rheumatic fever.

Additional Indexing Words:

Anti-streptokinase Anti-streptodornase Rheumatic fever Rheumatic heart disease Jones' criteria Streptococcal infection Streptococcus Glomerulonephritis Scarlet fever Antistreptolysin O

SINCE Jones' criteria for guidance in the diagnosis of rheumatic fever was revised in 1965,¹⁾ the necessity of the supporting evidence of preceding streptococcal infection has been accentuated and serological reactions to be added to the measurement of antistreptolysin O has been recommended, although they are uncommonly practised because of the technical complexity of the methods.

It is the purpose of this paper to present a new simple method for the measurement of anti-streptokinase-streptodornase (anti-SK-SD) in patient-sera and a preliminary report of its clinical applications.

METHODS

Erythrocytes: Human group O erythrocytes preserved in blood bank were used.

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Antigens: Varidase (Lederle Co., Ltd. No. 2200-1132-A) was used throughout the experiments. Only for the comparison Lot No. 2200-1141-A and Lot No. 2200-1129-AS were used.***

Formalinization of erythrocytes: Method described by Fauconnier²⁾ and modified by one⁵⁾ of us was used.

Hemagglutination technique: Boyden's tannic acid hemagglutination technique⁴⁾ modified by one³⁾ of us was used.

RESULTS

A. Fundamental studies:

(1) The investigation of influences of concentration of sensitizing antigens upon hemagglutinating titers (Table I and II). The concentration of one vial of Varidase diluted in 10 ml. of phosphate buffered saline was most suitable for the sensitization and this concentration was used throughout the experiments. The specificity of the reaction was evidenced by inhibition (Table III).

(2) The reproducibility of the hemagglutinating titers was evidenced as shown in Table IV. The formalin preserved erythrocytes sensitized with antigens were able to be used without changing the efficacy at least one month.

(3) The correlation between the hemagglutinating titers of anti-SK-SD and Todd units of anti-streptolysin O was observed (Table V). Titers of anti-SK-SD were usually higher than that of Todd units of anti-streptolysin O in the reciprocal sera.

Table I. Influence of Concentration of Antigen upon Titer of Hemagglutination (I)

Titer Concent. of Antigen	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	C
1 Vial/2.5 ml.	+	+	+	±	—	—	—	—	—
1 Vial/5 ml.	+	+	+	+	±	—	—	—	—
1 Vial/10 ml.	+	+	+	+	—	—	—	—	—
1 Vial/20 ml.	+	±	±	—	—	—	—	—	—
1 Vial/40 ml.	±	—	—	—	—	—	—	—	—

Lot. No. 2200-1132-A.....1 Vial: SK. 115,686 u. SD. 153,383 u.

*** These vials contain streptokinase and streptodornase as followings according to the data presented by Lederle (Japan), Ltd.

Lot 2200-1132-A	Lot 2200-1141-A	Lot 2200-1129-AS
SK 115,686 NIH u./vial	102,630 NIH u./vial	122,990 NIH u./vial
SD 153,383 NIH u./vial	150,866 NIH u./vial	87,975 NIH u./vial

Table II. Influence of Concentration of Antigen upon Titer of Hemagglutination (II)

Concent. of Antigen \ Titer	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1: 1,280	1: 2,560	C
1 Vial/10 ml.	+	+	+	+	+	+	+	+	±	—
1:2	+	+	+	+	+	+	+	±	—	—
1:4	+	+	+	+	+	+	+	±	—	—
1:8	+	+	+	+	+	+	±	—	—	—
1:16	+	+	+	±	±	—	—	—	—	—
1:32	+	±	±	±	—	—	—	—	—	—
1:64	±	—	—	—	—	—	—	—	—	—
C	—	—	—	—	—	—	—	—	—	—

Lot. No. 2200-1132-A.....1 Vial: SK. 115,686 u. SD. 153,383 u.

Table III. Hemagglutination Inhibition Reaction

Serum \ Antigen	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	C ₂
1:40	—	—	—	—	—	—	—	—
1:80	—	—	—	—	—	—	—	—
1:160	+	—	—	—	—	—	—	—
1:320	+	+	—	—	—	—	—	—
1:640	+	+	+	—	—	—	—	—
1:1,280	+	+	+	+	+	—	—	—
1:2,560	+	+	+	+	+	—	—	—
1:5,120	+	+	+	+	+	—	—	—
1:10,240	+	+	+	+	+	+	—	—
C ₁	+	+	+	+	+	+	—	—

Table IV. Reproducibility of Titer of Hemagglutination

Serum \ Date	30/III	1/IV	13/IV	15/IV	27/IV	28/IV	3/V	7/V
Serum No. 1	1:320	1:640	1:320	1:320	1:640	1:320	1:640	1:320
Serum \ Date		4/IV			27/IV			
Serum No. 2		1:160			1:160			
Serum \ Date		11/IV					3/V	
Serum No. 3		1:320					1:320	

(4) There is no correlation between titers of anti-SK-SD and C.R.P. (Table VI).

B. Clinical Studies :

(1) The distribution of titers in various disease groups was investigated in 183 patient-sera (Table VII). The titers were often extremely high in sera of patients suffering from rheumatic fever, acute glomerulonephritis, scarlet fever and chronic tonsillitis. The titers were usually low in sera of

Table V. Anti-SK·SD and ASLO

ASLO A-SK·SD	<100	100	166	250	500	625	833	1,250	2,500≤
≥1:10,240				○		○	○○○	○ ○○○○○	○○○○
1:5,120		○	○	○	○		○		
1:2,560		○○		○○ ○○○○○	○	○	○○	○	
1:1,280	○	○○○	○○	○○○○○ ○○○○○	○	○○○○○	○○○○○ ○○○○○	○	
1:640	○○○	○ ○○○○○	○	○○○ ○○○○○	○	○○			○
1:320	○○○ ○○○○○	○○○○○ ○○○○○	○○○○○	○○ ○○○○○					
1:160	○○ ○○○○○ ○○○○○	○○○○○		○					
1:80	○○○○○	○○○							
≥1:40	○○○ ○○○○○	○		○					

Total 153 sera

Table VI. Anti-SK·SD and C.R.P.

C.R.P. A-SK·SD	(-)	(+1)	(+2)	(+3)	(+4)
≥1:10,240				○	
1:5,120	○ ○				○
1:2,560	○ ○ ○				
1:1,280	○ ○○○○○				○ ○ ○ ○ ○
1:640	○ ○ ○ ○ ○		○ ○	○	
1:320	○○ ○○○○○	○	○	○○	
1:160	○			○	○
1:80	○ ○ ○ ○				○
≥1:40	○ ○				

Total 47 sera

patients suffering from other miscellaneous diseases and of apparently healthy persons (donors in blood bank).

(2) The hamagglutination of anti-SK-SD seemed us likely to be useful as one of the supporting evidences of preceding streptococcal infection in guidance for the diagnosis of rheumatic fever (Table VIII).

Table VII. Titers of Anti-SK-SD Hemagglutination in Various Disease Groups

Disease	Titer	<40	80	160	320	640	1,280	2,560	5,120	10,240 ≤	Total
Rheumatic Fever		1				3	3		3	3	13
Rheumatoid Arthritis			1	2	3	4		1			11
Tonsillitis		3	1	2	6	6	12	4		2	36
Rheumatic Heart Disease		1	2	3	3	2	6	1	1		19
Acute Nephritis						1	2		1	3	7
Chronic Nephritis		1	1		1	1	1				5
Scarlet Fever		2			2		2	2			8
Anaph. Purpura						1	1			1	3
Other Diseases		7	2	12	8	1					30
Gravidity				1	1	1	1				4
App. Healthy Persons		4	11	12	17	1	2				47

Total 183 cases

Table VIII. Rheumatic Fever

Patient	Symptoms and Findings	Major Manifestations			Minor Manifestations							Evidences of Strep. Infection		
					History	Pain	Fever	Acute Phase Reaction			E.K.G. PQ	Throat Culture	ASL-O	A-SK-SD
		Carditis	Polyarthr.	Chorea				ESR 1 hr.	CRP	W.N.				
T.O. 10y♂		A.I.	+	—	—	+	38°C	46	—	12,000	0.20	— Staphyl.	1,250	1 : 640
E.N. 13y♀		Syst. Mur. at Apex	+	—	—	+	39°C	60	—	9,600	w.n.l.*	± Strept.	2,500 ∧	1 : 20,480
M.O. 13y♀		Syst. Mur. at Apex	+	—	+	+	39.5°C	137	+	10,900	w.n.l.	—	2,500	1 : 10,240
T.A. 12y♀		—	+	+	—	+	37.5°C	274	+	9,800	w.n.l.	—	625	1 : 1,280
A.C. 6y♂		Syst. Mur. at Apex	+	—	—	—	37.5°C	80	+	13,200	w.n.l.	— Cand.	625	1 : 1,280

* within normal limits

Table IX. Rheumatoid Arthritis

Patient	Symptoms and Finding	Morning Stiffness	Joint Pain	Swelling of Joint	Affect. of Other Joint	Symmetrical Affection of Joints	X-ray of Joints	Rh-factor (R.A. Rose)	ASL-O	A-SK-SD
S.O.	70y♂	+	+	+	+	+	Stage 1	$\begin{matrix} + & 16 \\ \downarrow & \\ - & 4 \end{matrix}$	500	1:640
S.W.	51y♀	+	+	+	+	+	Stage 3	+ 16	250	1:640
T.I.	76y♀	+	+	+	+	+	Stage 3	$\begin{matrix} + & 8 \\ & 4 \end{matrix}$	<100	1:160
T.K.	27y♀	+	+	+	+	+	Stage 4	+ 16		1:320
H.M.	46y♀	+	+	+	+	+	Stage 4	+ 64	<100	1:160
M.M.	47y♀	+	+	+	+	+	Stage 1	+ 32	250	1:320
M.W.	46y♀	+	+	+	+	+	Stage 3	+ 32	100	1:80
S.Y.	52y♀	+	+	+	+	+	Stage 3	+	<100	1:320

Table X. Scarlet Fever

Case No.	Day of Disease	ASLO	Anti-SK-SD
1	3	250	1:320
2	3	250	1:2,560
3	5	250	1:2,560
4	5	250	1:320
5	10	100	<1:40
6	12	<100	<1:40
7	12	100	1:640
8	19	100	1:1,280

(3) The titers of anti-SK-SD were not high in sera of patients suffering from definite and classical rheumatoid arthritis (Table IX). This data suggests that rheumatoid arthritis may have less intimate relationship with streptococcal infection than rheumatic fever, as already reported by many investigators and us.

(4) Titers of anti-SK-SD were relatively higher than that of Todd units of antistreptolysin O in sera of 6 in 8 cases of scarlet fever (Table X).

DISCUSSION

Varidase⁶⁾ is a commercial product containing streptokinase and strepto-

dornase prepared from streptococcus group C and has been used as antigens in classical methods for the measurement of anti-streptokinase in patient sera, for example as Christensen's⁷⁾ method. Recently, the cross-reactivity between streptokinase of streptococcus group C and that of streptococcus group A has been discussed by many investigators,^{8), 9), 11), 12)} and Dilton and Wannamaker¹¹⁾ have reported that there exists the antigenic similarity between streptokinase of group C and most parts of streptokinase of group A. Therefore, it may be reasonable to consider that the titers of the hemagglutination reported in this paper represent the quantities of anti-streptokinase in patient-sera, although anti-streptodornase may influence the titers in sera containing much of anti-streptodornase, which may be rare by Kwapirsky¹⁰⁾ and by Wannamaker,^{13), 14)} who reported three distinct streptodornase A, B and C and varidase containing only streptodornase A.

It may be not so difficult to separate streptokinase and streptodornase and to measure anti-streptokinase and anti-streptodornase separately, using this hemagglutination technique. But, for the practical purpose of supporting evidence of preceding streptococcal infection, it may be superior to measure the higher one of the titers of anti-streptokinase and that of anti-streptodornase in patient-sera than to measure these two antibodies separately, because both these extracellular antigens participate simultaneously in practical streptococcal infection and, most of all, it is convenient to use easily attainable commercial product as antigens for the sensitization of erythrocytes in hemagglutination technique.

REFERENCES

1. Stollerman, G. H., et al.: *Circulation* **32**: 664, 1965.
2. Fauconnier, B.: *Ann. Inst. Past.* **95**: 777, 1958.
3. Kumagai, N.: *Nisshin-Igaku* **47**: 396, 1960 (in Japanese).
4. Boyden, S. V.: *J. Exp. Med.* **93**: 106, 1951.
5. Kumagai, N., et al.: *Acta Haemat. Japon.* **26**: 434, 1963 (in Japanese).
6. Bangham, D. R. and Walton, P. L.: *Bull. Wld. Hlth. Org.* **33**: 235, 1965.
7. Christensen, L. R.: *J. Clin. Invest.* **128**: 163, 1949.
8. Kaplan, M. H.: *Proc. Soc. Exp. Biol. Med.* **63**: 50, 1946.
9. Weinstein, L.: *Proc. Soc. Exp. Biol. Med.* **83**: 689, 1953.
10. Kwapirsky, J. B. and Synder, M. L.: *Immunology of Rheumatism*, Appelton-Century-Crofts, New York, p. 111, 1962.
11. Dilton, H. C. and Wannamaker, L. W.: *J. Exp. Med.* **121**: 351, 1965.
12. Houba, V. and Hana, I.: *Immunology* **11**: 387, 1966.
13. Wannamaker, L. W.: *J. Exp. Med.* **107**: 797, 1958.
14. Wannamaker, L. W.: *Am. J. Med.* **27**: 567, 1959.