

Circulating Immune Complexes in Korean Hemorrhagic Fever —Hemorrhagic Fever with Renal Syndrome—

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Abstract—To clarify the presence and the role of immune complexes in the pathogenesis of Korean hemorrhagic fever, circulating immune complexes were measured by platelet aggregation test, C1q-gel diffusion test, and polyethylene glycol precipitation test in the blood from the 24 patients during the early phase of Korean hemorrhagic fever. Serum complement and immunoglobulins were also measured. In all patients, circulating immune complexes were detected by one or more test. Detection rate of circulating immune complexes was 100% in the early phase and gradually decreased in the late phase. During the early phase of Korean hemorrhagic fever, the level of C3 decreased significantly and the level of immunoglobulin M increased from the fifth day of illness. Positive rate of circulating immune complexes were significantly correlated to decreased C3 level and severity of disease. It was concluded that circulating immune complexes play important roles in the pathogenesis of Korean hemorrhagic fever.

Key Word: *Korean hemorrhagic fever, Circulating immune complex, Platelet aggregation test, C1q-gel diffusion test, Polyethylene glycol precipitation test.*

INTRODUCTION

Korean hemorrhagic fever known as hemorrhagic fever with renal syndrome, is an acute infectious disease caused by Hantaan virus (Lee *et al.* 1978; Lee *et al.* 1981). Pathogenesis of Korean hemorrhagic fever are not clearly delineated to date. Recently, the immunologic mechanisms of Korean hemorrhagic fever have been investigated by many authors (Kang *et al.* 1976; Chang *et al.* 1979; Kim *et al.* 1972, 1976; Kim *et al.* 1975; Jokinen *et al.* 1977, 1978; Yang *et al.* 1981; Gu *et al.* 1984). Dai Zyng *et al.* (1981) and Penttinen *et al.* (1981) reported the presence of circulating immune complexes during the course of the epidemic hemorrhagic fever and nephropathia epidemica respectively. Both disease were found to be causally or virtually linked to same virus (Lee *et al.* 1979; Svedmyr *et al.* 1979; Lee *et al.* 1980).

Various tests for the detection of circulating immune complexes have been widely used in the stu-

dies of chronic disorders of unknown etiology and pathogenesis (McClusky 1978; Theofilopoulos 1980). However many available tests for the detection of circulating immune complexes are entirely specific for antigen antibody complexes, furthermore that, each test has its own source of error due to a various interfering factors. Therefore, several methods based on different principles should be performed to investigate the role of the circulating immune complexes in the pathogenesis of certain disorders (Agnello *et al.* 1981; Barnett *et al.* 1979; Pussel *et al.* 1978; Ritzman *et al.* 1982; Mannik *et al.* 1982; Williams *et al.* 1980). Until now there has been no reported study about the presence and role of circulating immune complexes in Korean hemorrhagic fever.

The purpose of this study is to elicit the immunological mechanism in general as well as the particular role of circulating immune complexes in the pathogenesis of Korean hemorrhagic fever through sequential measurements of circulating immune complexes by the three different tests; the platelet aggregation test (Penttinen *et al.* 1977), C1q gel

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diffusion test (Agnello *et al.* 1970) and polyethylene glycol precipitation test (Dugeon *et al.* 1977). Immunoglobulin levels and complement were also measured by single radial immunodiffusion (Mancini *et al.* 1960).

MATERIALS AND METHODS

Twenty four male patients with Korean hemorrhagic fever were studied. They were between 24 and 27 years of age. Diagnosis was confirmed by typical clinical courses and detection of the antibody to Hantaan virus by the indirect immunofluorescence method. Controls consisted of forty eight "normals", i.e; those without history of Korean hemorrhagic fever. Serial samples according to the day of illness were collected daily from the onset until twelfth day and once in the convalescent phase. The sera were stored at -70°C for platelet aggregation test, C1q gel diffusion test and at 4°C with sodium azide for polyethylene glycol precipitation test.

1. C1q gel diffusion test

1) Preparation of C1q

C1q was isolated by modified method described by Agnello from recalcified plasma by precipitation with calf thymus deoxyribonucleic acid (DNA) (Calbiochem Co. 2618) (Agnello *et al.* 1969).

Pooled recalcified plasma is dialyzed for 48 hours against 0.025M Veronal-0.1 M EDTA buffer (pH 8.6). Dialyzed serum is spun at $1,000\times g$ for 30 minutes at 4°C and then $25\ \mu\text{g}$ per ml of serum of calf thymus DNA is added and stirred for three hours and kept 36 hours at room temperature. The solution is spun at $16,000\times g$ for 30 minutes and the precipitate is washed four times with 0.05 M phosphate buffer (pH 6.9) and then 0.1 ml of deoxyribonuclease (1 mg/ml, Sigma Co. D-0751) is added to per ml of suspension. The suspension is stirred at 37°C for 3 hours and then dialyzed against DNase buffer (pH 6.9) for 24 hours.

The solution is spun at $100,000\times g$ for 30 minutes. Supernatant fluid is further purified by gel filtration on G-200 Sephadex in 0.3 M phosphate buffer, C1q is present in the exclusion volume.

Fractions containing C1q are precipitated by adding 28% $(\text{NH}_4)_2\text{SO}_4$ solution. The precipitate after 5 hours at 4°C is collected, dissolved in 0.1 M phosphate buffer (pH 7.0) and dialyzed overnight.

Undissolved precipitate is removed by spinning at $100,000\times g$ for 30 minutes.

The clear solution is divided into portions and stored at -70°C . The preparation was used at 0.2

mg/ml in diffusion plate.

2) Gel diffusion

Agarose (Behring Co. OREO-15) was dissolved in 0.025 M phosphate-0.01 M EDTA buffer (pH 7.2) to be 0.6% concentration and poured just before use.

Twenty two ml of agarose solution pipetted into a 9 cm petri dish to give a 5 mm thick and gel wells were cut 9 mm in diameter with center to center distance 12 mm.

$150\ \mu\text{l}$ of C1q solution was added in the center well and in the surrounding wells.

$150\ \mu\text{l}$ of specimens to be tested and aggregated human globulin as controls were added, and plates were incubated at 22°C for 48 hours.

2. Platelet aggregation test

1) Separation of platelet

320 ml of human blood (O blood group) is centrifuged at $1,700\times g$ for 2 and 1/2 minutes. And obtained plasma is centrifuged at $450\times g$ for 7 minutes, The sediments were discarded and the platelet rich plasma is collected and a third centrifugation is done at $1,900\times g$ for 30 minutes.

The excess plasma is transferred to another bag except for 20 ml in which the platelets are resuspended, and centrifuged for 20 minutes at $1,600\times g$, and the platelet form a white pellet.

The platelets were washed twice with normal saline and once with phosphate buffered saline (pH 7.5), and centrifuged for 15 minutes at $1,600\times g$. The final pellets is suspended in basal salt solution. After preliminary counting in a hemocytometer a photometer was used to adjust the concentration to $2\times 100,000$ platelets/ mm^3 .

2) Platelet aggregation test

Sera were diluted in BSS starting with a dilution of 1:2 to 1:128 in plastic U-microplates. To $25\ \mu\text{l}$ of serum dilution are added $25\ \mu\text{l}$ of BSS and $50\ \mu\text{l}$ of platelet suspension. The results were read by naked eye in dark field illumination after overnight incubation at 5°C .

A smooth white button indicates that the platelets have not reacted.

With each of the following controls are put up;

- 1) negative serum, unheated
- 2) known positive human immune complex serum.

3. Polyethylene glycol precipitation

$300\ \mu\text{l}$ of serum was diluted 1/25 in 4.8 ml of 0.1 M borate buffer (pH 7.4) and mixed in equal portion (5 ml) with PEG (PEG 6000) solution at final PEG concentration of 3.5%.

The mixture was then incubated for 18 hours at

4°C followed by centrifugation at 20,000×g for 20 minutes.

After decanting the supernatant, precipitates were dissolved in 5 ml of 0.1 N sodium hydroxide and the quantity of precipitated proteins present was measured by optical density (O.D.) at 280 nm.

4. Immunoglobulin, complement C3, C4.

For the determination of the levels of immunoglobulin and complement, single radial immunodiffusion method described by Mancinii was used.

Statistical analysis

The student t-test was used on data for comparing the level of immunoglobulin and complement. Chi-square test performed for evaluating the correlation between the various clinical parameters and positive rates of CIC and correlation of positivity among the different methods.

RESULTS

1. Circulating immune complexes in Korean hemorrhagic fever.

In normal control group of 48 healthy persons, titer of circulating immune complexes determined by platelet aggregation test was below 1:2 in all cases except for one where the titer was 1:4. Accordingly titers above 1:4, were interpreted as positive results. We can not observe any precipitation lines in all sera assayed by C1q gel diffusion test. Mean optical densities of tested samples measured by polyethylene glycol precipitation test were 0.105 ± 0.027 (mean \pm SD) and so optical densities above 0.16 were judged to be positive for circulating immune complexes.

During the whole course of the disease positive rates of circulating immune complexes were 91.8% (22 of 24) by platelet aggregation test, 58.3% (14 of 24) by C1q gel diffusion test, 77.8% (14 of 18) by polyethylene glycol precipitation test respectively. Among the 18 patients who were tested by all three methods, 15 patients (83.3%) were positive for circulating immune complexes by one or more methods. But there were no statistically significant correlations among positivity for circulating immune complexes by different methods (Table 1,2, Figure 1).

2. Circulating immune complexes according to the day of illness

As a whole, in samples obtained during an early period, positive rates were relatively high such as 100% in the third day but it decreased gradually to be under 20% during the convalescent phases. Ti-

Table 1. Positive rates of circulating immune complexes in Korean hemorrhagic fever

	Normal value	No. of patients		
		Total	Positive	rate(%)
PAT*	< 1:2	24	22	91.8
C1q**	no precipitation	24	14	58.3
PEG***	< 0.16	18	14	77.8

*PAT: platelet aggregation test

**C1q: C1q gel diffusion method

***PEG: polyethylene glycol precipitation method

Table 2. Correlations among the different methods in the positive rates of circulating immune complexes

Method	PAT		PAT	PAT		C1q	C1q	
	Posi-	Nega-		Posi-	Nega-		Posi-	Nega-
Result	tive	tive	tive	tive	tive	tive	tive	
Posi- tive	10	11	16	15	7	25	3	4
Nega- tive	32	23	6	2	3	4	3	4
χ^2	0.33		0.62		0.45			
p-value	>0.05		>0.05		>0.05			

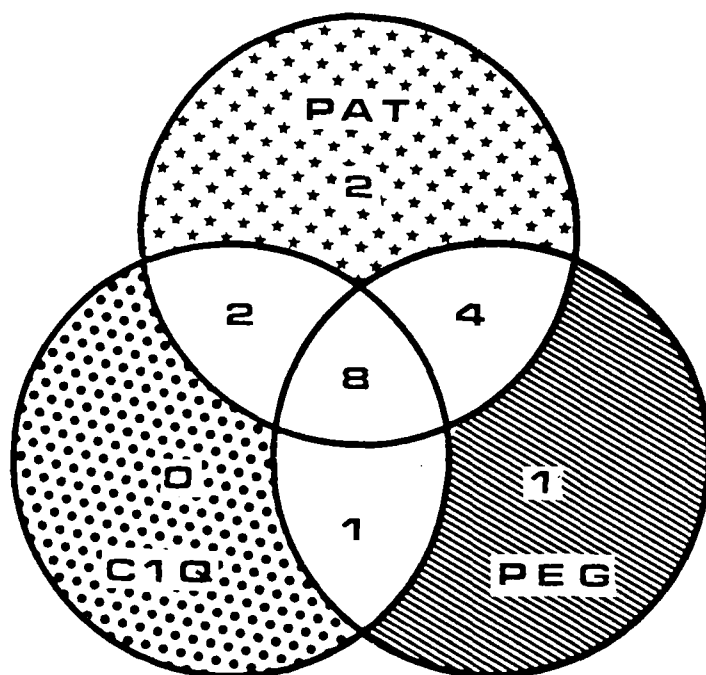


Fig. 1. Diagram of overlapping positive rates of circulating immune complexes in 18 patients with Korean hemorrhagic fever.

ters of circulating immune complexes by platelet aggregation test were high and the precipitation lines of C1q gel diffusion test were prominent in samples of the early period (Table 3, Figure 2,3,4).

3. Changes of immunoglobulin level and complement

Values of immunoglobulin G, M and A of normal controls were $1,032 \pm 38.0\text{mg/dl}$ (mean \pm SEM), $190 \pm 12.0\text{mg/dl}$, $91.0 \pm 4.0\text{mg/dl}$ respectively. IgM decreased significantly from the 5th day of illness and persisted until 12th day of illness. IgG level did

Table 3. Sequential changes of positive rates of circulating immune complexes (%)

Method	Day of Illness										C#
	3	4	5	6	7	8	9	10	12		
PAT*	100	86	72	42	50	30	38	50	45	8	
Clq**	100	50	60	25	25	25	13	0	20	13	
PEG***	100	100	75	67	67	100	100	50	50	20	

#C: convalescent period

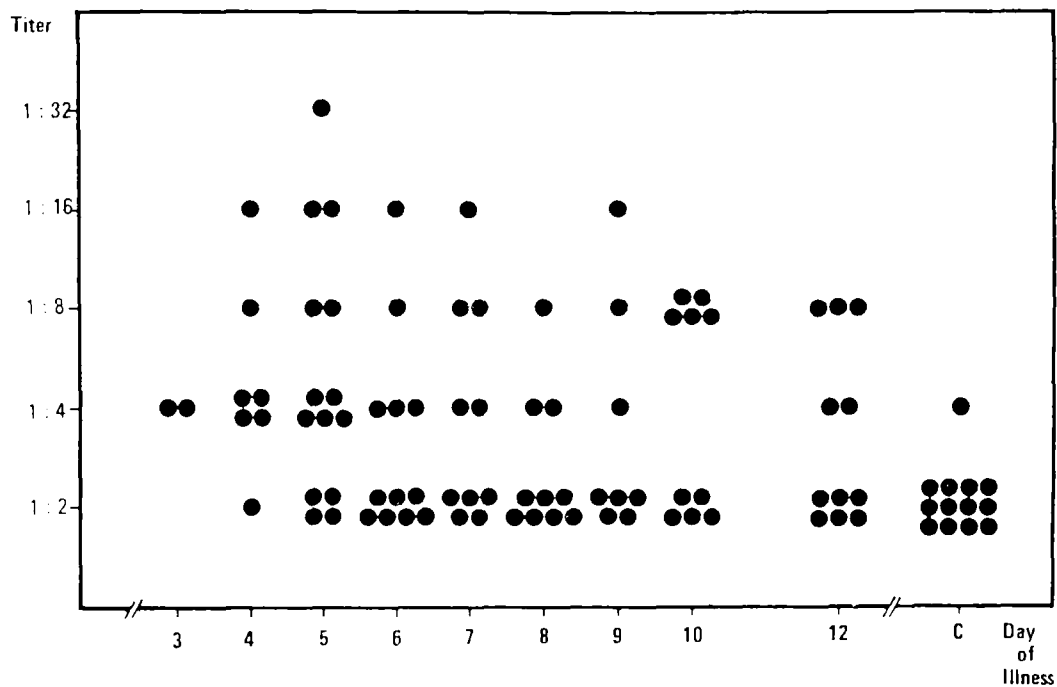


Fig. 2. Sequential changes of circulating immune complexes by platelet aggregation test. (C:convalescent period)

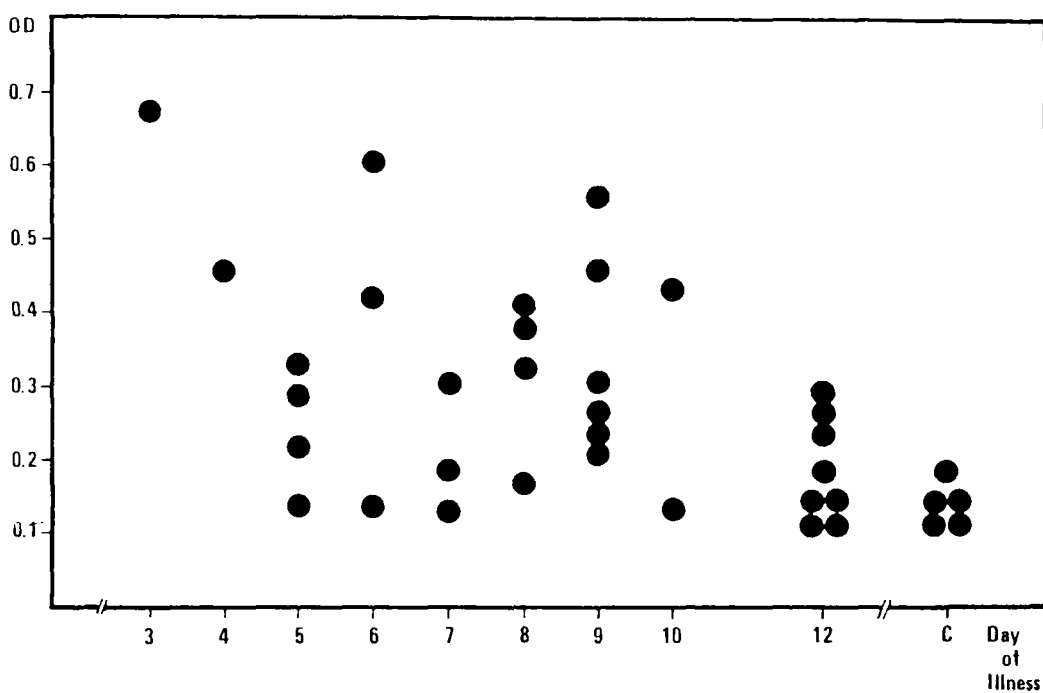


Fig. 3. Sequential changes of circulating immune complexes by polyethylene glycol precipitation method. (C:convalescent period)

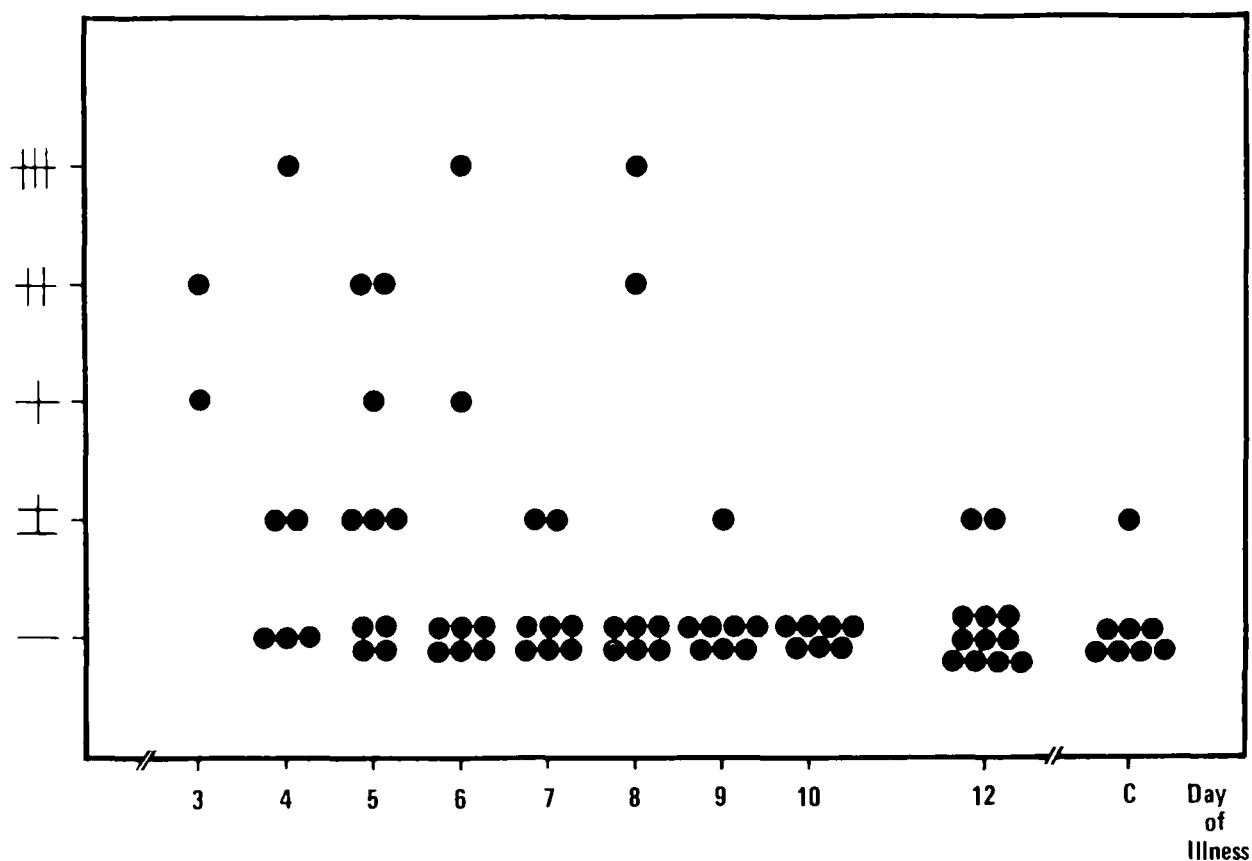


Fig. 4. Sequential changes of circulating immune complexes by Clq-gel diffusion method. (C:convalescent period)

Table 4. Sequential changes of immunoglobulin level

Immuno- globulin(mg/dl)		Day of Illness										Normal control
		3	4	5	6	7	8	9	10	12	C [#]	
Immunoglo- bulin G	Mean	1304	824	826	892	927	939	896	948	1088	1239*	1032
	±SEM	347.8	221.7	101.1	111.9	128.3	48.9	79.4	68.5	179.4	92.4	38.0
Immunoglo- bulin A	Mean	223	143	192	217	198	178	201	218	210	233	190
	±SEM	41.6	21.8	26.4	27.4	25.7	19.2	29.3	35.9	40.3	35.3	12.2
Immunoglo- bulin A	Mean	96	102	111*	122**	116**	109	124**	127*	114**	115	91
	±SEM	14.0	4.9	7.0	7.7	8.2	12.2	4.9	8.7	4.9	12.0	4.0

*: p<0.05

** : p<0.01

[#]C: convalescent period

not change in the early period but markedly increased in the convalescent phase (1,239 ± 92.4mg/dl). IgA level showed no significant changes during the whole course of the disease (Table 4).

Normal values of C3, C4 were 67.7 ± 1.8mg/dl (Mean ± SEM) and cut off values for normal C3, C4 was 43mg/dl, 4.9mg/dl which are below the mean

by two standard deviation.

In 12 out of 24 patients C3 decreased below the cut off values in one or more tested sera obtained before the 8th day, and mean value of C3 also decreased to be 49.0 ± 8.8mg/dl, 47.0 ± 5.4mg/dl on 4th, 5th, 6th days of illness respectively (Table 5).

The value of C4 did not decrease under cut off

Table 5. Sequential changes of complement level

Complement(mg/dl)		Day of Illness										Normal control
		3	4	5	6	7	8	9	10	12	C#	
Complement 3	Mean	57	49*	47**	56*	64	60	77	86	98**	99**	67.7
	±SEM	23.1	8.8	5.0	5.4	3.7	6.5	6.2	8.9	7.7	12.2	
Complement 4	Mean	12.7	9.8	9.0	11.2	9.0	11.4	11.1	12.4*	12.7*	10.3	10.3
	±SEM	2.2	0.7	0.9	0.7	1.2	1.4	0.6	0.9	1.3	0.4	

*: p<0.05

** : p<0.01

#C: convalescent period

values in any case but slightly decreasing tendency was noticed in early phase and significantly increased after the 10th day.

4. Correlation between circulating immune complexes and changes of complement level

Decreased C3 level and positive circulating immune complexes by platelet aggregation test were matched in 50 samples among 77 samples ($X^2=4.745$, $P<0.05$). The correlation was statistically significant. Results obtained by C1q gel diffusion test were matched 36 out of 61 samples and there were also significant correlation ($X^2=8.996$, $P<0.01$). However in case of polyethylene glycol precipitation test, there were no correlation (Table 6).

5. Correlation between circulating immune complexes and severity of diseases

During the course of the disease, patients with 3 or more of following situations; hypotension for more than 24 hours, oliguria for more than 5 days, clinically significant bleeding, more than 100mg/dl of BUN, and the case requiring hemodiaysis are classified as severe group and others are classified

as mild group. Between the severe and mild group, positive rates of circulating immune complexes measured by C1q gel diffusion test were shown to be significantly different, but differences were not significant by other methods (Table 7).

6. Correlation between the positivities of circulating immune complexes and day of illness

Table 6. Correlations between circulating immune complexes and complement level

CIC#		Complement 3 level		Significance
		decreased	normal	
PAT*	Positive	15	19	p<0.05
	Negative	8	35	$x^2=4.745$
C1q**	Positive	10	11	p<0.01
	Negative	4	36	$x^2=8.996$
PEG***	Positive	3	15	p>0.05
	Negative	1	10	$x^2=0.000$

#: circulating immune complex

Table 7. Positive rates of circulating immune complexes according to clinical severity

CIC	Group	Severe			Mild			Significance
		Total	Positive	%	Total	Positive	%	
PAT*		39	19	48.7	48	20	41.6	p>0.05#
C1q**		25	14	56.0	32	7	21.9	p<0.01##
PEG***		14	12	85.7	23	13	56.5	p>0.05###

#: $x^2=0.194$

##: $x^2=5.634$

###: $x^2=2.183$

¹⁾Clinically severe group: 3 of the following 5 items; hypotension>24hr, oliguria >5 days, BUN>100mg/dl, clinical bleeding tendency, treated with hemodialysis.

Differences in positive rates of circulating immune complexes before and after the 8th day, that is the last day up to which decreased C3 from the onset of illness, was observed, was significant by platelet aggregation test and C1q gel diffusion test (56.4% vs 33.3%, $X^2=4.195$, $P>0.05$) (40.5% vs 12.1%, $X^2=6.031$, $P<0.05$). But polyethylene glycol precipitation test was not (81.3% vs 57.1%, $X^2=1.434$, $P>0.05$) (Table 8).

DISCUSSION

It is dengue hemorrhagic fever that immunological mechanisms are definitely elucidated to play important roles in the pathophysiology of viral hemorrhagic fever (Borkisch *et al.* 1973; Bull WHO 1973; Boonpucknavig *et al.* 1976; Mitrakul *et al.* 1977; Rosen *et al.* 1977; Ruanjurachuporn *et al.* 1979). Presence of circulating immune complexes are proven in patients blood and renal tissues and etiologic viral antigen and antibody are isolated from the circulating immune complexes. Therefore it is considered as an immune complex mediated disease (Ruanjurachuporn *et al.* 1979).

In Argentin hemorrhagic fever, complement activation was reported but presence of circulating immune complexes was not confirmed (Maiztegu *et al.* 1975; Maria *et al.* 1978).

In nephropathia epidemica and epidemic hemorrhagic fever in China, the disease are known to be caused by same or similar virus of Korean hemorrhagic fever (Lee *et al.* 1979; Svedmyr *et al.* 1979; Lee *et al.* 1980), circulating immune complexes was identified but pathophysiologic significance was not proven (Dai *et al.* 1981; Penttinen *et al.* 1981). The chinese method of circulating immune complexes measurement was confined only to platelet aggregation test. Therefore one may not insist that circulating immune complexes plays major role in pathogenesis of these viral hemorrhagic fevers.

According to several previous reports, the possibility of immunologic role in the pathogenesis of Korean hemorrhagic fever has been suggested but not confirmed (Kang *et al.* 1976; Chang *et al.* 1979; Kim *et al.* 1972, 1976; Kim *et al.* 1975). According to our results by platelet aggregation test, on the 3rd, and 4th day of illness the positive rate of circulating immune complexes were 100%, and 86% respectively, and rapidly decreased to 8% during the convalescence. The above findings strongly suggested the presence of circulating immune complexes. The forth, causative virus of nephropathia epidemica and Korean hemorrhagic fever are very similar but not same (Svedmyr *et al.* 1980). Specific character of virus is also possible cause of differences.

And to insist the role of circulating immune complexes in clinical process, production must be correlated to severity of disease or duration and activation of complement. But all above data from China (Dai *et al.* 1981) and Scandinavia (Penttinen *et al.* 1981) did not include informations related to clinical severity or complement activation. Even though our data showed that poor correlation to clinical severity, complement activation has good correlations.

The results of C1q gel diffusion test also showed much difference between the early and convalescent phase (100% vs 18%) and totally 14 out of immune complexes during the early course of Korean hemorrhagic fever and agree to the fact of a 100% detection rate of circulating immune complexes during the 3 to 7 day period of illness reported by Chinese author, Dai *et al.* (1981). But there are many differences in the detection rate as reported by Penttinen *et al.* (1981), the 22% positive rate in nephropathia epidemica. The causes of this much differences are supposed first to be that the nature

Table 8. Positive rates of circulating immune complexes before and after the 8th day of illness

Group	Severe			Mild			Significance
	Total	Positive	%	Total	Positive	%	
CIC							
PAT*	55	31	56.4	42	14	33.3	p>0.05#
C1q**	42	17	40.5	33	4	12.1	p>0.05##
PEG***	16	13	81.3	21	12	57.1	p>0.05###

#: $x^2=4.195$

##: $x^2=6.031$

###: $x^2=1.434$

of circulating immune complexes in two diseases are different. Secondly samples of Penttinen's data were gathered in a relatively later period. This finding corresponded to the fact that our results obtained in a late phase also revealed low positive rate. The third, defect of platelet aggregation test lower positive rate of circulating immune complexes in the presence of rheumatoid factor. Even we didn't check rheumatoid factor, high positive rate of rheumatoid factor of Penttinen's data is one possible 24 (58.3%) were positive. Positive rate of circulating immune complexes by C1q gel diffusion test in Korean hemorrhagic fever has not been reported yet. And the rate, only in nephropathia epidemica, was reported to be 29% by C1q binding radioimmunoassay. In dengue hemorrhagic fever, which is different from Korean hemorrhagic fever, the positive rate by the agglutination method using C1q and IgG coated latex, was 84% and it can be assumed that it has a correlation with the severity and duration of the disease (Ruanjirachuporn *et al.* 1979).

In this study, positive rate by C1q gel diffusion test assay was observed to have statistically significant relationship with the severity, duration of disease and complement activation. These observations point to conclusions that the circulating immune complexes detected by C1q gel diffusion test may play the very important role to clinical manifestations.

Several methods for the detection of immune complexes by C1q isolation have been devised. The DNA precipitation technique in this study was less productive in C1q yield than the other methods. And C1q gel diffusion test also has pitfalls in both the quantitative determination of circulating immune complexes and interpretation of precipitation lines. We believe our present results should be further explored using more sensitive methods such as C1q binding radioimmunoassay or C1q deviation test.

Positive rate by polyethylene glycol precipitation test decreased from 100% in the initial phase to 20% in convalescent phase. However, it was different in its results from that by foregoing two methods and remained high in the late stage.

Although detection of circulating immune complexes by polyethylene glycol precipitation test in Korean hemorrhagic fever has not been reported, the positive rates of circulating immune complexes by conglutinin binding radioimmunoassay was maintained longer in nephropathia epidemica than

that by platelet aggregation test or C1q binding assay (Penttinen *et al.* 1981). Therefore the high positive rates demonstrated in the later stage was considered to be due to polyethylene glycol precipitation test itself. This assumption was further supported by the finding that positive rates of circulating immune complexes by polyethylene glycol precipitation test had no correlation with the severity and duration of the disease, and was not accompanied by C3 decrease, but influencing factors on the positive rate such as immunoglobulin itself in polyethylene glycol precipitation test or reaction by nonspecific protein should also be considered. However numbers of tested samples were so few to be statistically meaningful.

As Lamber *et al.* (1978) reported the positive rate of circulating immune complexes in same disease state, had a low correlation with individual method, our data also showed no significant correlation among the different methods.

It has been reported that there was a significant rise of IgM in nephropathia epidemica, but IgG and IgA exhibited normal levels (Penttinen *et al.* 1981).

Previous Chinese study also reported that levels of IgM were significantly high but those of IgG and IgA were not (Yang *et al.* 1981). Lee *et al.* (1976, 1980) described that IgM reached maximum levels within 8 to 10 days after onset and that IgG progressively rose to peak levels within 25 to 30 days after onset. In this study, we found that levels of IgM rose from the 5th day after onset to reach a maximum by 10 to 12 days. And IgG in contrast to IgM showed no statistically significant changes at initial stage but significant rise at convalescent stage, IgA did not show any significant changes throughout the course of the disease. These results were consistent with those reports by Lee *et al.* (1976, 1980).

In studies of the activation of complement system, Kang *et al.* (1976) and Chang *et al.* (1979) observed that C3 was primarily activated. They suggested that activation of the alternative pathway might play a major role. However Yang *et al.* (1981) and Gu *et al.* (1984), in their extensive studies of activation of the complement system in China, reported that they could find complement activation in patient's sera at the initial stage of epidemic hemorrhagic fever and so suggested both complement pathway could be activated. Mainly complement pathway was shown to be activated by both, classical and alternative pathways in 50% of cases tested. And in case of complement activation

by only one pathway, in 30.5% of patients was shown to be activated by classical pathway and in 2.5% by alternative pathway respectively. Their results indicated that complement was shown to be primarily activated by classical pathway. Our data indicated that C3 level was reduced in 12 of 24 patients without significant reduction of C4 level in all cases. These findings highly suggested activation of alternative pathway. Incidences of decreased C3 level were significantly correlated to the positive rate of immune complexes, and C4 level was significantly elevated during the convalescent phase. So C4 activation also could be predicted. In this regard we considered that both pathways were activated in Korean hemorrhagic fever.

In summary, immune complexes are formed in the early period of Korean hemorrhagic fever and positive rates of circulating immune complexes have significant correlation with the severity, courses of disease and decrease of complement levels. So it is suggested that circulating immune complexes plays important roles on the pathogenesis of Korean hemorrhagic fever. Studies for the clarification of characters of immune complexes should be performed by further analysis of this complexes in the future.

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= 국문초록 =

한국형출혈열에서의 혈중 면역복합체에 관한 연구

한림대학 의학부 내과학교실

박정식

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한국형출혈열의 면역학적 특성 내지는 면역복합체의 동태를 규명하고자 한국형출혈열 환자 24명을 대상으로 혈소판 응집법, C1q-gel diffusion법 및 polyethylene glycol 침전법으로 혈중 면역복합체를 측정함과 동시에 면역 글로불린 및 혈청보체를 병일에 따라 측정하여 다음과 같은 결과를 얻었다.

1. 한국형출혈열 환자 전예에서 측정법에 따라 양성율에는 차이가 있으나 혈중 면역복합체의 존재가 증명되었으며 측정법과 혈중 면역복합체 양성을 간에는 유의한 상관관계가 없었다.
2. 질병 경과 중 혈중 면역복합체 양성율은 초기 100%에서 서서히 감소하여 질병 후에는 20%이하로 감소하였다.
3. 혈중 면역복합체가 높은 양성율을 나타내는 초기에 혈청보체 C₃의 감소와 IgM의 현저한 증가가 관찰되었다.
4. 혈소판 응집법 및 C1q-gel diffusion법으로 측정한 혈중 면역복합체 양성율과 혈청보체 C₃의 감소는 유의한 상관관계를 나타냈다.
5. C1q-gel diffusion법으로 측정한 혈중 면역복합체는 질병의 중증도와 유의한 상관관계를 보였다.

이상의 성적을 종합하면 한국형출혈열에 있어서는 초기에 혈중 면역복합체가 생성되며, 이는 임상상과 연관성이 있음을 밝혀 한국형출혈열의 병태생리에 혈중 면역복합체가 관여함을 밝혔다.