A PEDIGREE OF DEFICIENCY OF THE NINTH COMPONENT OF COMPLEMENT (C9)

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Summary Findings in a large family with a hereditary deficiency of the ninth component of complement (C9) were reported. The proposita was a 64-year-old Japanese woman with gastric cancer. C9 was undetectable by both rocket immunoelectrophoresis and hemolytic assays. Despite the C9 deficiency her serum showed half the normal level of hemolytic complement activity (16 CH50 U/ml). C9 was not detectable in her two sisters who had no underlying disease. Other complement components were of normal levels in these two sisters. In 8 males and 7 females from three generations of the family, levels of C9 protein were reduced and ranged from 22 to 46% of the normal. Such corresponds to heterozygote for the mutant gene, and this defect may be due to autosomal incompletely dominant gene.

The C9 deficient individuals were not liable to specific diseases.

INTRODUCTION

The hereditary defects heretofore reported mostly involved the classical pathway of complement components (Alper and Rosen, 1979; Agnello, 1978). Since the report of the first case of C9 deficiency by Inai *et al.* (1979), other occurrences in Japan have also been documented (Kisu *et al.*, 1980; Yukiyaama *et al.*, 1979; Amano *et al.*, 1980; Kawano *et al.*, 1980).

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We now report the chemical and immunological findings in members of a large family with C9 deficiency.

MATERIALS AND METHODS

Blood samples taken from the family members allowed to clot at room temperature, and the sera were stored at -80°C .

Complement assays: The total hemolytic activity of complement in serum (CH50) was measured by the method of Mayer (1961) using only 1/2.5 the volume of the reagents (Nagaki *et al.*, 1965). Hemolytic activities of serum complement components were measured using microtiter plate assay (Nelson *et al.*, 1966) and appropriate intermediate cells and reagents respectively. Purified human C9, a commercial product from Cordis Laboratories, Inc., Miami, U.S.A., was used for the restoration test of hemolytic activity in the C9 deficient serum, and anti-C9 rabbit antiserum (Behringwerke AG, Marburg, West Germany) for measurement of C9 protein by rocket immunoelectrophoresis. Anti-C9 antiserum was added to the buffered agarose gel (Agarose A45, Nakarai chemical Co., Japan) at 3% of final concentration, and the electrophoresis were performed for about 18 hr with a potential drop of 2 v/cm.

Serum protein concentrations of C3 and C4 were measured by radial immunodiffusion using a commercially available immunodiffusion plate (Partigen, Behringwerke AG, Marburg, West Germany).

RESULTS

Titration of hemolytic activities of serum complement components

In the proposita and her two sisters, as shown in Table 1, hemolytic activities of C9 were not detectable, though those of other complement components, C1 to C8, were within normal limits in the latter two. In the proposita's serum, however, hemolytic activities of C4, C2, C3, C5, C6, and C7 were also depressed, suggesting that other factor(s) affects the hemolytic activities of these components in the serum of the proposita, as a carcinoma-bearing host. Amounts of C4 and C3 proteins were normal (Table 3). The absence of C9 protein in these sera was also confirmed by the method of single radial immunodiffusion and rocket immunoelectrophoresis (Kisu *et al.*, 1980). Hemolytic activities of C9 in sera of two brothers of the proposita were reduced to 1/4 and 1/8 of that in normal human serum, respectively, the other components being within normal ranges.

Restoration of the whole hemolytic activity by the addition of isolated C9 in the C9 deficient sera

As shown in Fig. 1, various amounts of functionally pure C9 (0.04–100 U/ml) were added to C9 deficient sera from two sisters (II-3 and II-15) for restoration test,

Table 1. Hemolytic activities of serum complement components in the C9 deficient members of the family.

Deficiency state	Case number	Hemolytic activities of serum complement components								
		C1	C4	C2	C3	C5	C6	C7	C8	C9
Homozygous deficiency	II-3	800	3,200	1,600	1,600	3,200	12,800	6,400	6,400	<200
	II-6	6,400	<200	<200	<200	<200	<200	<200	1,600	<200
	II-15	1,600	1,600	1,600	800	3,200	3,200	1,600	1,600	<200
Heterozygous deficiency	II-10	1,600	3,200	800	800	3,200	3,200	1,600	1,600	400
	II-17	3,200	800	1,600	1,600	3,200	6,400	3,200	6,400	200
No deficiency	II-8	1,600	3,200	800	400	6,400	3,200	800	3,200	1,600
	II-13	1,600	6,400	1,600	800	800	3,200	1,600	3,200	800
Healthy control		3,200	3,200	3,200	1,600	1,600	6,400	6,400	12,800	1,600

Fig. 1. Restoration of serum complement hemolytic activity by adding various concentrations of isolated C9 in the C9 deficient serum. ●, ■, homozygote (II-3 and II-15 in Fig. 2, respectively); ○, healthy control.

and the results in full restoration of the hemolytic activity were obtained by addition of more than 6.25 U/ml of C9, but no sign of restoration was evident in the case of the proposita's serum.

Restoration of C9 hemolytic activity by the addition of isolated C9 in the C9 deficient sera

C9 hemolytic activity in the C9 deficient sera was measured using EAC1-8 intermediate cells. Isolated C9 of various concentrations were added to a mixture

Table 2. Restoration of C9 hemolytic activity by adding various concentrations of isolated C9 in the C9 deficient serum.

	Amounts of isolated C9 added (U/ml)											
	100	50	25	12.5	6.25	3.13	1.57	0.79	0.40	0.20	0.10	0
II-3	4	4	4	3	2	2	1	1	0	0	0	0
	4	4	4	3	2	1	1	0	0	0	0	0
II-6	3	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0
II-15	4	4	4	3	2	2	1	1	0	0	0	0
	4	4	3	2	2	1	1	0	0	0	0	0
Healthy control	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4

0, 1, 2, 3 and 4 showing degree of hemolysis; 4: complete lysis, 0: no lysis. Test for restoration duplicated on each C9 deficient serum.

of EAC1-8 cells and C9 deficient sera. As shown in Table 2, addition of 25 to 100 U/ml of functionally pure C9 to sera from C9 deficient siblings (II-3 and II-15) resulted in full restoration of the hemolytic activity, and addition of 0.79 to 12.5 U/ml of C9 resulted in partial restoration of hemolytic activity, suggesting that there were no inhibitory factor(s) to C9 hemolytic activity in these sera.

On the other hand, addition of C9 of relatively high concentrations partially restored the hemolytic activity in the proposita's serum, indicating that some inhibitory factor(s) for C9 were present in the proposita's serum.

Family studies

Table 3 shows the levels of CH50, C3, C4, and C9 in sera from members of the kindred. In the C9-deficient individuals, the values of CH50 were only slightly reduced and ranged from 16 to 23 U/ml, those of C3 and C4 proteins being within normal ranges.

Fifteen individuals of the family showed reduced C9 levels, comprising 22 to 46% of the value of a pooled serum from 450 healthy adults (standard serum). These findings indicate that these individuals possessed heterozygous state for the C9 mutant gene. In these heterozygotes C3 and C4 as well as CH50 were within normal limits, except III-13. The family tree is shown in Fig. 2.

Individual tests of 337 sera from healthy adults were performed in order to investigate the normal range of C9 protein by rocket immunoelectrophoresis and expressed as a percentage of the concentration of C9 protein in the standard serum.

The distribution of C9 values in sera from healthy adults was shown in Fig. 3. The mean value and standard deviation (1S) was equal to $95.8 \pm 26.2\%$ of the C9

Fig. 2. Pedigree of the C9 deficient family. ●, homozygote (completely deficient); ■, heterozygote (partially deficient); □, normal value for C9.

Table 3. Values of CH50, C3-, C4- and C9 protein in the family members of C9 deficiency.

	CH50 (U/ml)	C3 (mg/dl)	C4 (mg/dl)	C9 (%)
II- 3**	16	109	60	0
II- 4	52	80	37	98
II- 6**	23	115	80	0
II- 8	40	78	54	84
II-10*	42	78	54	35
II-13	66	90	50	95
II-15**	16	95	54	0
II-16	55	75	52	98
II-17*	52	101	62	35
III- 1*	60	98	43	36
III- 3*	60	86	44	22
III- 5*	52	86	44	38
III- 7*	42	86	60	32
III- 8*	46	75	39	34
III- 9*	NT	NT	NT	33
III-12*	NT	NT	NT	33
III-13*	23	70	44	42
III-18*	30	64	38	36
III-20	29	88	41	96
III-22	39	90	56	120
III-23	36	82	34	75
III-24*	70	110	77	46
III-25*	39	86	34	34
IV- 1	37	112	37	68
IV- 2*	39	119	30	39
IV- 3	37	112	36	65
IV- 4*	45	147	62	39
IV- 7	32	125	58	80

** Homozygous deficiency, * heterozygous deficiency. C9, 100%=250 pooled NHS; NT, not tested.

Fig. 3. Distribution of C9 protein levels in healthy adults and proposed-heterozygous C9 deficient individuals. 100%=pooled normal human serum (n=450). ●, proposed-heterozygous C9 deficient individual.

value in the standard serum. Nine of 337 healthy adults (2.7%) showed low C9 level less than half of the standard serum.

Closed circle in Fig. 3 represented the serum C9 values of individuals assumed to be heterozygous for C9 deficiency.

DISCUSSION

Hereditary defects of complement components reported thus far mostly involved the classical pathway in human, including C1r (Pickering *et al.*, 1971), C1s (Pondman *et al.*, 1968), C4 (Hauptmann *et al.*, 1974), C2 (Klemperer *et al.*, 1966), C3 (Ballow *et al.*, 1975), C5 (Rosenfeld *et al.*, 1976), C6 (Leddy *et al.*, 1974), C7 (Boyer *et al.*, 1975; Gelâge *et al.*, 1977; Nemerow *et al.*, 1978), and C8 (Petersen *et al.*, 1976), and two inactivators of the complement system, C1 inhibitor (Donaldson and Evans, 1963), and C3b inactivator (Abramson *et al.*, 1971; Thompson and Lachmann, 1977). Few cases of ninth component (C9), the terminal component of the complement sequence, have been reported.

Inai *et al.* (1979) were the first to report a case of deficiency of C9 in a 29-year-old healthy woman, and seven families with C9 deficiency have recently been detected in Japan (Kisu *et al.*, 1980; Yukiyaama *et al.*, 1979; Amano *et al.*, 1980; Kawano *et al.*, 1980). Lint *et al.* (1980) recently reported such an occurrence in a Caucasian man. These findings suggest that the frequency of C9 deficiency may not be so rare. The probanda of this study, a 64-year-old woman, was first identified to be C9 deficient since the precipitating anti-C9 antibody was found in the serum after having received the blood transfusions (Okochi and Inaba, 1980). Despite the deficiency of C9, the probanda's serum showed half the normal hemolytic activity.

Studies on all family members revealed that two sisters were also completely deficient in C9 and fifteen members had levels of less than half of the normal. The former two would correspond to individuals homozygous for the mutant gene, as was the probanda, and the latter fifteen to heterozygous ones. This defect may be due to an autosomal, incompletely dominant gene. Level of protein was zero in homozygous individuals, and less than half the normal in heterozygous individuals, the levels of C9 protein ranging from 22 to 46% of the normal. Thus, levels of C9 protein in serum of normal homozygote (C_9, C_9), heterozygote ($C_9, \bar{C}_9$), and deficient homozygote ($\bar{C}_9, \bar{C}_9$) approximately reflected the dosage effect of the wild type and mutant gene. Some of the known defects of complement components in man, including C2 (Klemperer *et al.*, 1966; Fu *et al.*, 1974; Day *et al.*, 1975), C3 (Alper and Rosen, 1979), C5 (Rosenfeld *et al.*, 1976; Snyderman *et al.*, 1979), C6 (Leddy *et al.*, 1974; Lim *et al.*, 1976; Glass *et al.*, 1978), C7 (Boyer *et al.*, 1975; Gelâge *et al.*, 1977; Nemerow *et al.*, 1978), and C8 (Petersen *et al.*, 1976) appear to be inherited in the same mode as is C9. One of the well documented exceptions to this mode of inheritance is the deficiency of C1 inhibitor (Donaldson and Evans, 1963), causative pathogenesis of hereditary angioedema. In the studies of inherited C6 deficiency

Glass *et al.* (1978) inferred that a low level of serum C6 in individuals with a C6 deficiency results from the heterozygous state of normal C6 variant gene and a silent or null C6 gene. Pariser *et al.* (1978) reported that hereditary C2 deficiency was determined by the same mechanisms involved in the deficiency of C6.

Consanguineous marriage was not identified in the past few generations of the proposita.

None of C9 deficient individuals in this family appear to be liable to some specific ill conditions as a result of this deficiency, excepting that the untoward proposita died with a gastric cancer, as did others with a complete C9 deficiency (Inai *et al.*, 1979; Amano *et al.*, 1980; Kawano *et al.*, 1980). It was reported that only one male showed a tendency to have repeated infections (Yukiyama *et al.*, 1979) and a child had an anamnesis of anaphylactoid purpura (Kawano *et al.*, 1980).

Lint *et al.* (1980) reported that C9 deficiency was not linked to the HLA-A or B antigens. We obtained no evidence suggesting a close linkage between C9 deficiency and major histocompatibility loci (Sato, 1980). Also, there was no linkage between C9 deficiency and the blood group system including ABO, Rh, Lutheran, Kell, MNS, P, Duffy, Kidd, Diego, and Xg (Otsuka, 1980).

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