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HUMAN C7 POLYMORPHISM: QUANTITATIVE ANALYSIS OF DIFFERENT PHENOTYPES

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Summary C7 polymorphism was investigated in a Japanese population (Tokyo). The C7 protein concentrations and hemolytic C7 activities in the serum samples of various phenotypes were measured by radial immunodiffusion (RID) and radial diffusion hemolysis (RDH). The mean C7 protein levels for the common phenotypes C7 1, C7 2-1, C7 3-1, and C7 4-1 were 88%, 103%, 63%, and 94% of a standard, while the mean C7 activity levels were 96%, 90%, 63%, and 74%, respectively. Both the protein and the activity levels for C7 3-1 were significantly lower than those determined for the most common phenotype C7 1. The mean levels in two individuals of C7 3 phenotype were only 34% in protein concentration and 47% in functional activity. Concerning the ratio of functional to immunochemical C7 (*i.e.* the relative specific activity), there was no difference between the phenotypes C7 1 and C7 3-1. On the other hand, the mean hemolytic activity and the relative specific activity for C7 4-1 were significantly lower than those for C7 1.

INTRODUCTION

The complement component C7 is known to be one of the precursors of the membrane attack complex in the complement system. Genetic polymorphism of human C7 was first described by Hobart *et al.* (1978) in a Caucasian population. The authors demonstrated three codominantly expressed alleles at an autosomal locus: one common allele, *C7*1*, and two rare alleles, *C7*2* and *C7*3*. In contrast, in Japanese four different alleles, *C7*1*, *C7*2*, *C7*3*, and *C7*4*, have been observed

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at polymorphic frequencies (Tokunaga *et al.*, 1986; Washio *et al.*, 1986); the *C7*5* described by Nishimukai and Tamaki (1986) corresponds to *C7*3* (Washio *et al.*, 1986).

These four alleles were also observed in two Mainland Chinese populations (Zeng *et al.*, 1986). Moreover, two rare variants have been reported, one in a Chinese individual (Zeng *et al.*, 1986), and the other in a Japanese individual (Washio *et al.*, 1986). It is interesting that both C6 (the sixth component of complement) and C7, which are tightly linked each other (Hobart *et al.*, 1978; Tokunaga *et al.*, 1986), show a higher degree of polymorphisms in East Asian populations than in Caucasians.

It has been suggested that *C7*3* is a 'hypomorphic' variant: Hobart *et al.* (1978), who revealed the C7 phenotypes by isoelectric focusing and a functional hemolytic detection, reported that the C7 3 bands are usually weaker than the common C7 1 bands. A similar observation was made by Nishimukai and Tamaki (1986) and Washio *et al.* (1986) using an immunoblotting procedure for the detection of C7 banding patterns.

The purpose of the present study is to determine antigenic and functional C7 levels in relation to the different phenotypes of C7.

MATERIALS AND METHODS

Serum samples. Serum samples were collected from 201 healthy unrelated donors living in Tokyo. The samples were stored at $-80°C$ and tested within a few months.

Identification of C7 allotypes. Typing for C7 was performed as described previously (Washio *et al.*, 1986). Briefly, after treatment with neuraminidase, desialized serum samples were subjected to isoelectric focusing in thin layer polyacrylamide gels followed by an immunoblotting procedure. For a small number of desialized samples of various phenotypes, a functional detection was performed by means of a C7 specific hemolytic overlay.

Immunochemical C7 quantitation. Serum samples of various phenotypes were subjected to radial immunodiffusion (RID) in 1.2% agarose gel containing 3.3% anti-human C7 serum (Cappel) and 0.1% NaN_3. The C7 protein levels were expressed as percentages of a standard serum pool. The standard serum pool was a mixture of equal volumes of seven C7 1 samples, two C7 2-1 samples, one C7 4-1 sample and one C7 3-1 sample, and used in three dilutions on each plate.

Functional C7 quantitation. C7 hemolytic activities were measured by radial diffusion hemolysis (RDH) in 0.8% agarose gels containing 1.9×10^8 antibody-coated sheep erythrocytes (EA)/ml and 2% homozygous C7 deficient human serum (kindly provided by Prof. C. Rittner). C7 hemolytic activities were expressed as percentages of the standard serum pool.

Statistical analysis. Statistical significance of the differences in C7 levels between the various phenotypes was tested by Mann-Whitney's U test.

RESULTS

Figure 1 demonstrates the C7 phenotypes that were observed in this study. After isoelectric focusing of neuraminidase-treated serum samples, C7 was identified either antigenically by immunoblotting (Fig. 1a) or functionally by hemolytic overlay (Fig. 1b). It can be seen that in both detection systems the C7 3 bands are considerably weaker than the C7 1 bands.

Table 1 shows the results of C7 typing in 201 adult Japanese. The allele frequencies estimated for *C7*1*, *C7*2*, *C7*3* and *C7*4* were 0.803, 0.085, 0.072 and 0.040, respectively. The observed numbers of phenotypes were in good agreement with those expected on the assumption of Hardy-Weinberg equilibrium ($\chi^2=4.20$, 5 d.f., $0.5<p<0.7$).

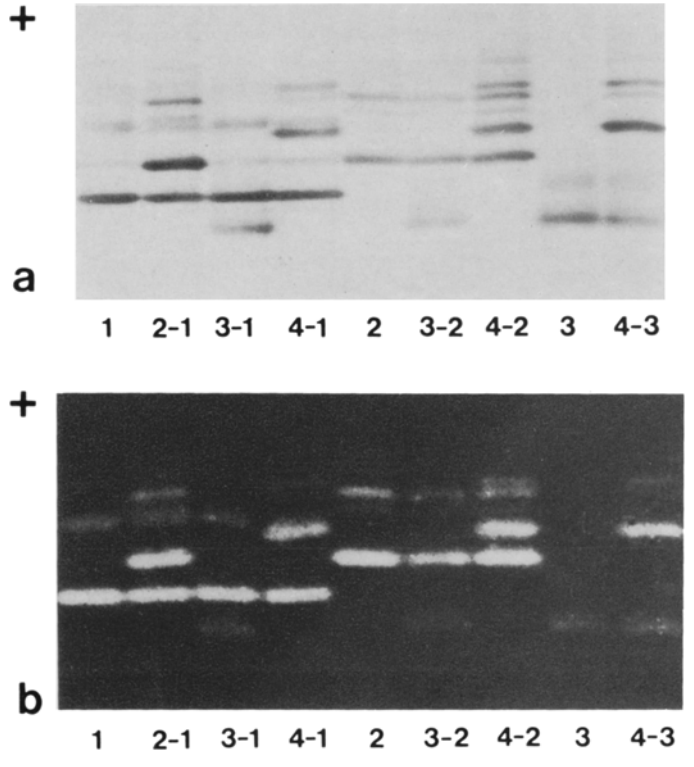

Fig. 1. Patterns of C7 phenotypes obtained by isoelectric focusing followed by (a) immunoblotting or (b) hemolytic overlay.

Table 1. Distribution of C7 phenotypes and allele frequencies in a Japanese population (Tokyo).

Phenotypes	No. observed	%	No. expected	Allele frequencies
1	133	66.2	129.6	*C7*1*=0.803
2-1	23	11.4	27.4	
3-1	21	10.4	23.2	*C7*2*=0.085
4-1	13	6.5	12.9	
2	3	1.5	1.5	*C7*3*=0.072
3-2	3	1.5	2.5	
4-2	2	1.0	1.4	*C7*4*=0.040
3	2	1.0	1.0	
4-3	1	0.5	1.2	
Others	0	0	0.3	
	201	100.0	201.0	1.000

χ^2=4.20, 5 d.f., $0.5<p<0.7$.

Table 2. C7 protein and hemolytic activity levels for various phenotypes.

Phenotypes	No. of samples tested	Protein level (%) mean±SD	Activity level (%) mean±SD	Ratio of activity/protein mean±SD
Common phenotypes				
1	21	88±22	96±16	1.14±0.30
2-1	20	103±25	90±22	0.95±0.38
3-1	20	63±13	63±16	1.01±0.23
4-1	12	94±27	74±12	0.86±0.30
Rare phenotypes				
2	3	90	92	1.08
3	2	34	47	1.38
3-2	3	85	105	1.26
4-2	2	121	85	0.71
4-3	1	70	85	1.21

C7 protein level

Table 2 shows the results of the immunochemical C7 quantitation for four common and five rare phenotypes. The mean levels for the common phenotypes C7 1, C7 2-1, C7 3-1, and C7 4-1 were 88% (SD=22%), 103% (SD=25%), 63% (SD=13%), and 94% (SD=27%), respectively. Thus, the mean C7 protein level for C7 2-1 is slightly higher than that for C7 1 ($p<0.05$) and that for 3-1 is considerably lower than that for C7 1 ($p<0.001$) as shown in Table 3. Two individuals of C7 3 phenotype showed the lowest values with a mean of 34%.

Table 3. Comparisons of C7 levels by Mann-Whitney's U test.

	Probability		
	Protein level	Activity level	Ratio
C7 1 *vs.* C7 2-1	<0.05	NS [a]	<0.05
C7 1 *vs.* C7 3-1	<0.001	<0.0001	NS
C7 1 *vs.* C7 4-1	NS	<0.001	<0.01

[a] NS, not significant.

C7 functional level

The mean hemolytic C7 level for each phenotype is also presented in Table 2. The mean values for the common phenotypes C7 1, C7 2-1, C7 3-1, and C7 4-1 were 96% (SD=16%), 90% (SD=22%), 63% (SD=16%), and 74% (SD=12%), respectively. The mean level of hemolytic C7 activity for individuals of the C7 3-1 phenotype is significantly lower than that for individuals of the C7 1 phenotype ($p<0.0001$), thus corresponding to the result of immunochemical quantitation (Table 3). On the other hand, the activity levels for individuals of C7 4-1 phenotype are significantly lower than those for individuals of C7 1 phenotype ($p<0.001$), although there is no difference in the protein levels (Table 3). Individuals of C7 3 phenotype again showed the lowest values with a mean of 47%.

Relative specific activity

The relative specific activity (the hemolytic efficiency) of a sample was defined as the ratio of functional to immunochemical C7, each expressed as a percentage of the same standard serum pool. The mean value for C7 3-1 samples (1.01) was similar to that for C7 1 sample (1.14). In contrast, the mean relative specific activity for C7 4-1 (0.86) was significantly lower than that for C7 1 ($p<0.01$). The value for C7 2-1 (0.95) was slightly lower than that for C7 1 ($p<0.05$) (Tables 2 and 3).

DISCUSSION

Previous studies on C7 polymorphism suggested that C7 3 might be a 'hypomorphic' variant (Hobart *et al.*, 1978; Nishimukai and Tamaki 1986; Washio *et al.*, 1986). Our present investigation demonstrates that both the immunochemical and functional C7 levels for C7 3-1 are lower than those for the other common phenotypes. The hemolytic efficiency of the protein coded by *C7*3* may fall within normal range, because for the phenotype C7 3-1 the ratio of functional to immunochemical C7 was close to that for the C7 1 phenotype. Therefore, the product of the *C7*3* allele is considered to be normal in hemolytic activity but reduced in quantity.

In agreement with the conclusion that the *C7*3* allele is associated with a decreased C7 serum concentration, the two individuals with the phenotype C7 3 ex-

hibited the lowest C7 levels. However, we could not demonstrate that these two individuals are really *C7*3/C7*3* homozygotes because family members for the analysis of the genotypes were not available for C7 typing. Akagaki *et al.* (1983) found five C7 deficient individuals among 52,000 blood donors from the Osaka area. Assuming that C7 deficiency is determined by a silent or null allele at the C7 structural locus, the frequency of the *C7*Q0* allele might be about 0.01 in the Japanese population. Thus, the underlying genotype for the two individuals of C7 3 phenotype could also be *C7*3/C7*Q0*, although it seems more likely that the low C7 levels in the C7 3 individuals are due to a *C7*3/C7*3* genotype, because of the rather high *C7*3* frequency in our present sample.

In contrast to *C7*3*, the product of *C7*4* may be normal in quantity but have a slightly lower activity. The samples of C7 4-1 phenotype showed a significantly decreased hemolytic activity, in spite of normal protein levels. The mean value of the relative specific activity for C7 4-1 samples was significantly lower than that for C7 1 samples.

The relationships between structural genetic phenotypes and serum levels have been studied for several other polymorphic complement components. In the C2 polymorphism, some variants might have a reduced hemolytic activity, whereas one variant (C2 AT) is associated with a significantly increased activity (Raum *et al.*, 1979; Tokunaga *et al.*, 1981). In the C3 system, a rare hypomorphic C3 variant (C3f, now C3 FQL) was found by Alper and Rosen (1971), while quantitative immunochemical as well as functional (hemolytic) measurements did not show a significant difference between phenotypes (Brönnestam, 1973; Colten and Alper, 1972). Extensive studies have been performed on the variable hemolytic activities of the difference C4 allotypes (see for example Dodds *et al.*, 1985). There are large differences in activity not only between C4A and C4B allotypes, but also between allotypes of the same locus. In the factor B (BF) polymorphism, the *BF*F* allele is associated with an antigenically and functionally higher BF serum concentration than the *BF*S* allele (Mauff *et al.*, 1980; Mortensen and Lamm, 1981); a rare hypomorphic variant (BF FQL), however, seems to result in a relatively low concentration in serum (Raum *et al.*, 1984).

Further studies have to be performed to analyze the structural basis of the C7 3 variant and the mechanisms for its decreased serum concentration. In addition, it will be interesting to investigate the possible biological significance of the observed quantitative differences between the various C7 allotypes as well as the possible associations with certain diseases.

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