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GENETIC POLYMORPHISM OF THE SECOND COMPONENT OF HUMAN COMPLEMENT (C2) IN JAPANESE

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Summary Polymorphism of the second component of human complement (C2) was investigated in a total of 292 sera from unrelated adult Japanese using a slab polyacrylamide gel isoelectric focusing followed by a specific hemolytic assay. Besides the common phenotype (C), two relatively infrequent double-banded phenotypes (AC and BC) were observed, which were considered to be heterozygotes. The estimated frequencies for the common allele, $C2^C$, and the variant alleles, $C2^A$ and $C2^B$, were 0.937, 0.046, and 0.017, respectively. 229 samples were further typed for HLA-A, HLA-B, and HLA-C, and the result indicated the presence of significant association of $C2^A$ with *HLA-B15*. This finding suggests that in Japanese there is an allelic combination showing linkage disequilibrium between C2 and HLA loci which is different from those in Caucasians.

INTRODUCTION

The second component of human complement (C2) is one of the components of the classical complement pathway with a single polypeptide chain of M.W. 102,000 (Polley and Müller-Eberhard, 1968; Kerr and Porter, 1978).

Hereditary deficiency of C2 has been reported to be relatively common in Caucasian populations, the deficient gene frequency being approximately 1% (Agnello, 1978), while it is not thus far reported in Japanese. Close linkage between the C2 locus and the MHC region was observed in the families with homozygous C2 deficiency (Fu *et al.*, 1974; Day *et al.*, 1975).

Genetic polymorphism of human C2 and its linkage with HLA was recently discovered in Caucasian populations using isoelectric focusing (Hobart and Lachmann, 1976; Alper, 1976; Meo *et al.*, 1977). The polymorphism was shown to

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be controlled by codominant alleles at a single locus situated very close to HLA-D locus (Raum *et al.*, 1979; Dewald and Rittner, 1979). As to Asian populations, a single study on a very small sample of "Orientals" in USA has been published, though there was no detailed information concerning the racial status of the sample (Alper, 1976).

In this report, we present the result of the population study of C2 polymorphism in Japanese using a slab polyacrylamide gel isoelectric focusing followed by the specific hemolytic assay. Moreover, the HLA (A, B and C) typing were also carried out in order to examine the presence of linkage disequilibrium between C2 and HLA loci.

MATERIALS AND METHODS

A total of 292 sera or plasmas obtained from unrelated healthy adults living in Tokyo were used for population study of C2. Among these, 229 blood samples were also typed for the HLA (A, B and C) specificities. Samples for C2 typing had been stored at −40°C up to several weeks until used.

2.2% Ampholine (LKB) (6 parts pH 5–7 or 5–8, 1 part pH 3.5–10) was incorporated into 5.0% polyacrylamide gel (thickness 1 mm) with 0.2 M taurine (Alper, 1976). The gel was photopolymerized with riboflavin. After prefocusing at 600 V constant voltage for 1 h (electrode distance 11 cm), *ca.* 20 μl of undiluted samples were applied with pieces of filter paper at the anodal side of the gel, and the voltage was raised to 800 V. After 1 h, filter paper pieces were removed and the voltage was raised and kept constant at 1,000 V for 2.5–3.5 h.

The gel after focusing was washed in 5×10^{-5} M I_2/PBS (pH 6.0) for 10 min at 37°C in order to increase C2 activity (Polley, 1971; Olaisen *et al.*, 1978). The gel was then layered with 0.8% agarose gel (thickness 1 mm) in isotonic veronal buffered saline at pH 7.4 containing 0.1% gelatin, 1 mM $MgCl_2$ and 0.15 mM $CaCl_2$ (GVB^{2+}), 0.8% sheep erythrocytes sensitized with rabbit antibody (EA), and about 0.8% normal human serum instead of C2 deficient serum (Olaisen *et al.*, 1978). Hemolytic bands appeared after 2–4 h incubation at 37°C.

In an alternative method (Nishioka *et al.*, 1966), 0.8% agarose gel (thickness 1 mm) in GVB^{2+} containing 0.7% EAC14 intermediate cells was overlayed on the polyacrylamide gel. After 30 min of incubation at 37°C, C2 bands were developed by further overlaying 0.5% agarose gel containing 10% fresh guinea pig serum with 0.01 M EDTA in veronal buffered saline. The EAC14 intermediate cells were obtained by sensitization of sheep erythrocytes with rabbit antibody in 0.01 M EDTA contained gelatin veronal buffered saline, washing out of EDTA with 0.15 mM $CaCl_2$ contained gelatin veronal buffered saline (GVB-Ca^{2+}), and mixing of 20 parts of EA (2×10^9 cell/ml) in GVB-Ca^{2+} and 1 part of fresh guinea pig serum for 7.5 min at 0°C.

HLA (A, B and C) typing were carried out using peripheral blood lymphocytes by means of the standard microcytotoxicity technique.

Allele and haplotype frequencies, coefficients of linkage disequilibrium (D value), and its corresponding standard errors were estimated from the population data using the formulae presented by Mittal *et al.* (1972).

RESULTS

By both detection methods mentioned above, essentially the same lytic pattern for C2 was obtained (Fig. 1). Therefore, the hemolytic assay using normal human serum (Alper-Olaisen's method) which is simpler than that of Nishioka and others was used routinely in the present study. The common phenotype (C2 C) consisted of three prominent bands and a few additional bands. Two kinds of relatively infrequent variant phenotypes were found. They showed double-banded patterns, one with the variant C2 component at the acidic side, and the other with that at the basic side of the common C2 band. They are tentatively called type C2 AC

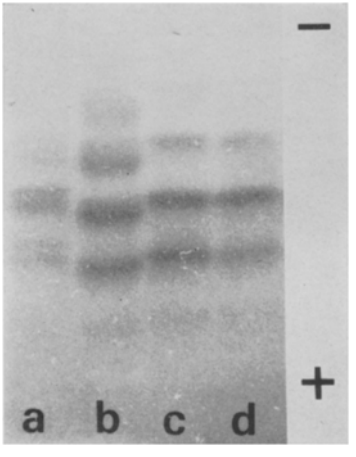

Fig. 1. Lytic patterns of C2 phenotypes obtained by isoelectric focusing and hemolytic assay. a, type C2 BC; b, type C2 AC; c and d, type C2 C.

Table 1. Distribution of phenotypes and allele frequencies of C2.

Total		Phenotype					
		C	AC	BC	A	AB	B
292	Obs. No.	255	27	10	0	0	0
	%	87.3	9.2	3.4	0	0	0
	Exp. No.	256.17	25.29	9.37	0.62	0.46	0.09

$\chi^2=1.335$, 3 d.f., $0.70<p<0.80$

Allele frequencies: $C2^C$ 0.937 ± 0.010, $C2^A$ 0.046 ± 0.009, $C2^B$ 0.017 ± 0.005

Table 2. HLA-A,B,C, and C2 allele frequencies estimated from 229 Japanese samples.

Allele	Frequency	Allele	Frequency	Allele	Frequency
A1	0.0044	*Bw44(12)*	0.0539	*Bw46*	0.0154
A2	0.2553	*B13*	0.0198	*Bw48*	0.0154
Aw24(9)	0.3525	*B15*	0.1184	*Cw1*	0.1184
A26(10)	0.1826	*Bw39(16)*	0.0492	*Cw2*	0.0088
A11	0.0867	*B17*	0.0044	*Cw3*	0.3133
Aw31	0.0820	*Bw22*	0.0220	*Cw4*	0.0378
Aw33	0.0539	*Bw54*	0.0655		
B5	0.2322	*B27*	0.0022	$C2^C$	0.930
Bw51	0.1012	*Bw35*	0.1061	$C2^A$	0.050
Bw52	0.1134	*B37*	0.0044	$C2^B$	0.020
B7	0.0492	*B40*	0.1907		

Table 3. HLA phenotypes of C2 AC and C2 BC individuals.

Individual	C2 phenotype	HLA phenotype					
1 Y.A.	AC	Aw24	Aw33	B40	Bw35	Cw1	
2 M.N.	AC	A26	Aw33	B15	B40	Cw3	
3 S.H.	AC	Aw24	A26	Bw35	B40	Cw3	
4 C.T.	AC	Aw24	A11	Bw51	Bw35	Cw3	
5 S.U.	AC	Aw24	A26	B15	Bw54	Cw1	
6 K.T.	AC	Aw24	Aw31	B7	B15		
7 M.K.	AC	Aw24	A26	B15	Bw35		
8 N.S.	AC	A26		Bw35	B40	Cw3	
9 T.I.	AC	Aw24		Bw52	B15	Cw1	
10 T.N.	AC	A2	Aw24	Bw52	B15	Cw3	
11 S.Y.	AC	Aw24	A26	B15	Bw54	Cw1	Cw3
12 G.S.	AC	A11	Aw31	Bw39	Bw54	Cw1	
13 K.S.	AC	Aw24	A26	Bw51	B15	Cw3	
14 M.A.	AC	A26	Aw31	B15	B40	Cw3	
15 M.H.	AC	A2	A26	B15	Bw35	Cw3	
16 K.K.	AC	A26		Bw52	B15	Cw3	
17 K.A.	AC	A2	A26	B15	Bw39		
18 Y.S.	AC	Aw24		Bw51	B7		
19 H.T.	AC	A2	Aw24	B7	B15		
20 M.I.	AC	A2	A26	B15	Bw54	Cw1	Cw3
21 S.N.	AC	A2		B15	B40	Cw3	
22 H.M.	AC	Aw24	Aw31	Bw51	Bw52	Cw1	
23 T.H.	AC	Aw31	Aw33	B12	Bw35		
24 G.T.	BC	Aw24		Bw51	Bw54	Cw1	
25 Y.Y.	BC	A2	Aw24	Bw35			
26 K.M.	BC	Aw24	A26	B7		Cw1	
27 A.I.	BC	Aw24	A11	Bw52	B40		
28 M.M.	BC	Aw24	A26	Bw52	B40	Cw3	
29 I.A.	BC	A2	Aw24	B40		Cw3	
30 M.O.	BC	A2	Aw24	Bw52	B40		
31 K.O.	BC	A26		Bw54		Cw1	
32 K.H.	BC	Aw24	A26	Bw51		Cw3	

Table 4. Estimates of haplotype frequencies (HF[a]) and linkage disequilibrium parameters (D) between *HLA* and *C2*

	HF	D	t
Aw24–C2^A	0.0169	−0.0013	−0.16
A26–C2^A	0.0216	0.0122	1.83
B15–C2^A	0.0315	0.0254	3.48[b]
Bw35–C2^A	0.0116	0.0061	1.13
Cw3–C2^A	0.0223	0.0062	0.83
Aw24–C2^B	0.0131	0.0061	1.33

[a] Only the haplotypes exceeding 0.01 are given. [b] Significance level: $p<0.001$.

and type BC, respectively, following the nomenclature used by Alper (1976). The pequliarity of the AC type is that the acidic side variant bands are apparently stronger than the common bands. As to type BC, the basic side variant bands have equal strength with the common bands.

The results of C2 typing of 292 unrelated samples are presented in Table 1. The allele frequencies for $C2^C$, $C2^A$, and $C2^B$ were estimated to be 0.937, 0.046, and 0.017, respectively. The distribution of C2 phenotypes was in good agreement with Hardy-Weinberg's equilibrium.

A total of 229 samples in the population material mentioned above were also typed for the HLA-A,B, and C specificities (Table 2). The C2 allele frequencies in this material agreed well with those in the whole material for C2 population study mentioned above. The results of HLA typing of 23 individuals of type C2 AC and 9 of type C2 BC are presented in Table 3. Among the C2 AC individuals, eighteen had either B15 or Bw35, and two had both B15 and Bw35. By the t-test on the C2 AC/HLA-A,B,C data, the positive association of $C2^A$ with *HLA-B15* was found to be statistically significant (Table 4). The linkage disequilibrium parameter of $C2^A/B15$ estimated from the present population data was: $D=0.0254$ ($t=3.48$, $p<0.001$). Nine C2 BC individuals were included, but no significant association between $C2^B$ and HLA alleles was detected.

DISCUSSION

The common C2 type observed in the present study (Fig. 1c and d) is considered to be identical with C2 C reported by Alper (1976) or C2 1 by European authors (Meo *et al.*, 1977; Olaisen *et al.*, 1978; Dewald and Rittner, 1979). Likewise, the basic double-banded type observed in Japanese (Fig. 1a) corresponds to C2 BC reported by Alper, or C2 2-1 by European authors. The frequency of $C2^B$ in Japanese (0.017) is lower than those in the Europeans and the American Caucasians (0.025–0.04).

The C2 "AC" type was first observed by Alper (1976) in the American Caucasians, and more recently, Pariser *et al.* (1978) described two rare acidic variants, C2 A1C and C2 A2C. At present, it is not certain as to whether the type AC discovered in the present study (Fig. 1b) is identical with one of the Caucasian variants. In any case, it is noteworthy that in Japanese $C2^A$ which is commoner than $C2^B$ has a polymorphic frequency of 0.046 (Table 1). Because no "AC" types have been observed in the Europeans the occurrence of these types in the American Caucasians raises an interesting question on the origin of these variants. Alper (1976) reported the C2 data in the Orientals (n=43), in which three cases of type C2 BC were found, but none of type C2 AC. However, the racial status of this "Oriental" sample was not given.

It is interesting that the significant positive association of $C2^A$ with *HLA-B15* ($p<0.001$) was found in the present study (Table 4). This finding is in contrast with the data reported in Caucasian populations, in which *B15* is in linkage disequilibrium with $C2^B$ ($C2^2$) (Meo *et al.*, 1976; Olaisen *et al.*, 1978; Raum *et al.*, 1979; Dewald and Rittner, 1979). Furthermore, two examples of C2 A1C in the American Caucasians were reported to show association with different HLA haplotypes, *A2, Bw17* and *A3, Bw21* (Raum *et al.*, 1979). Therefore, the $C2^A$ which is relatively common in Japanese population may have arisen by a mutation which occurred relatively recently in Japanese, or in some Mongoloid group. Further investigations of the distributions of C2 types and of C2/HLA association in other Mongoloid populations with known geographical origins are clearly needed.

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