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GENETIC POLYMORPHISM OF THE FOURTH COMPONENT OF HUMAN COMPLEMENT IN JAPANESE[1]

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Summary Polymorphism of the fourth component of human complement (C4) was investigated in Japanese using an agarose gel electrophoresis and a slab polyacrylamide gel electrophoresis followed by immunofixation. Three common phenotypes were observed corresponding to the types F, FS and S reported in Europeans. Typing of the family material consisting of 48 matings with 96 offsprings showed no discrepancy from the postulate that C4 polymorphism is controlled by a pair of codominant alleles. In a sample from 291 unrelated healthy Japanese, the allele frequencies of $C4^F$ and $C4^S$ are estimated at 0.543 and 0.457, respectively.

INTRODUCTION

Structural polymorphism of the fourth component of human complement (C4) was described by Rosenfeld *et al.* (1969) and Bach *et al.* (1971). They observed various C4 patterns using an antigen-antibody crossed electrophoresis in agarose gel, although it was not confirmed in family studies that the polymorphism is genetically controlled.

Teisberg *et al.* (1976, 1977) demonstrated a C4 polymorphism using an agarose gel electrophoresis followed by immunofixation, and by a specific hemolytic technique. They showed that the polymorphism was controlled by codominant alleles at a single locus, and that the C4 locus is situated in the region of major histocompatibility complex on chromosome 6 very close to the HLA-B locus. Linkage between HLA and C4 loci was also reported in families with C4 deficient patients (Rittner *et al.*, 1975; Ochs *et al.*, 1977). Mauff *et al.* (1978) reported the gene frequency data of C4 in a German sample using a modified agarose gel electrophoretic system.

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However, no population study of Asians including Japanese have thus far been carried out as to the C4 polymorphism.

The purpose of the present study is to confirm the genetically controlled polymorphism of C4 using a family material and to examine the distribution of C4 phenotypes in Japanese.

MATERIALS AND METHODS

The family material consisting of 48 matings with 96 children was obtained in Kamogawa City, Chiba Prefecture. The material for population study comprises two samples of unrelated, healthy adults: one from Chiba Pref. (N=103), and the other from Tokyo (N=188). Samples used for C4 typing were either heparinized plasma or the ACD-plasma to which at least 10 mM EDTA in the final concentration was added. The fresh plasma were used immediately for typing, or stored at −40°C until use.

Two distinct electrophoretic methods were employed for typing of C4 phenotypes. In the one method, a high voltage agarose gel electrophoresis was performed for 2.5–3 hr at 15 V/cm (4°C). A glass plate (180×180×1.2 mm) was covered with a 1.3 mm thick layer of 1.2%(w/v) agarose gel in a continuous 0.1 M Tris–0.04 M glycine buffer (pH 9.0). In the other method, a thin layer(thickness 1 mm) vertical slab polyacrylamide gel electrophoresis was carried out using the disc electrophoretic system by Ornstein and Davis (Davis, 1964) with slight modifications. The acrylamide concentration of the separating gel was 4.3%, and 0.08 M Tris–0.08 M borate–0.003 M EDTA buffer (pH 8.4) was used for the bridge buffer to prevent the conversion of C4.

Immunofixation (Alper and Johnson, 1969) was performed after the electrophoretic separation by overlaying antiserum on the gel for 1–1.5 hr at room temperature. The gel was then washed overnight (agarose) or 48 hr (polyacrylamide) with several changes of saline and then stained with 0.04% Coomassie Brilliant Blue G-250 in acetic acid-methanol-water(1:3:6) solution.

C4 protein was isolated from fresh human serum by sodium sulphate precipitation, DEAE cellulose chromatography, Sephadex G-200 gel filtration (Lachmann *et al.*, 1973), and further by preparative disc electrophoresis. This purified C4 showed a single protein band by polyacrylamide gel electrophoresis and immunoelectrophoresis at a concentration of approximately 10 mg protein/ml. Anti-C4 antisera were obtained from rabbits immunized with three injections of the purified C4 in complete Freund's adjuvant. The antiserum obtained showed a single arc of precipitate on immunoelectrophoresis of fresh human serum.

RESULTS

By both the agarose gel electrophoresis and the slab polyacrylamide gel electro-

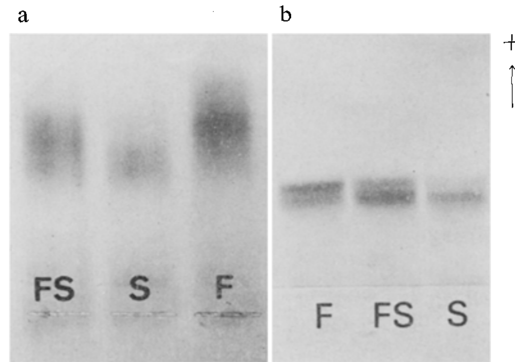

Fig. 1. Immunofixation patterns of three common C4 phenotypes after the agarose gel electrophoresis (a) and the slab polyacrylamide gel electrophoresis (b).

Table 1. C4 phenotypes in 48 families with 96 children.

Matings		No. of Children	C4 types of Children		
Type	No.		F	FS	S
F×F	3	7	7	—	—
F×FS	18	37	15	22	—
F×S	4	7	—	7	—
FS×FS	12	23	5	16	2
FS×S	9	18	—	9	9
S×S	2	4	—	—	4
Total	48	96	27	54	15

Table 2. Distribution of C4 phenotypes in the Japanese populations.

Populations	Phenotypes			Total	χ^2 (1 d.f.)	p	Allele frequencies
	F	FS	S				
Chiba							
No. obs.	30	54	19	103	0.379	0.70>p>0.50	$C4^F$: 0.553
No. exp.	31.6	50.9	20.5	103.0			$C4^S$: 0.447
Tokyo							
No. obs.	56	90	42	188	0.261	0.70>p>0.50	$C4^F$: 0.537
No. exp.	54.3	93.5	40.3	188.1			$C4^S$: 0.463
Total							
No. obs.	86	144	61	291	0.0025	p>0.95	$C4^F$: 0.543
No. exp.	85.8	144.4	60.8	291.0			$C4^S$: 0.457

phoresis, three phenotypes were observed: one mainly with the fast migrating bands (F), one mainly with the slow migrating bands(S) and the one with both bands(FS). In the agarose gel electrophoresis (Fig. 1a), each phenotype F or S consisted of about 3 major bands and a few minor bands, while the FS pattern consisted of about 6 bands

having almost the same intensity. In the polyacrylamide gel electrophoresis (Fig. 1b), F or S consisted of one major band, while FS of two bands.

In order to confirm the genetic basis of each C4 phenotype, a family study was performed (Table 1). The family material consisting of 48 matings with 96 children could be typed as one of the three phenotypes. The observed number of each mating type agreed with the number expected by random matings. The results supported the postulate that C4 polymorphism is controlled by a pair of codominant alleles, $C4^F$ and $C4^S$, at a single locus.

In a population study, two samples obtained from unrelated healthy adults were examined. In the sample from Chiba Pref. (N=103), allele frequencies for $C4^F$ and $C4^S$ were estimated at 0.553 and 0.447, respectively (Table 2). In the Tokyo sample (N=188), these allele frequencies were 0.537 and 0.463 (Table 2). The observed numbers of phenotypes were in good agreement with the numbers expected on the basis of Hardy-Weinberg's law. The difference in the allele frequencies between the two samples was statistically not significant ($\chi^2=0.124$, d.f.$=1$, $0.80>p>0.70$). Combined allele frequencies for $C4^F$ and $C4^S$ were 0.543 and 0.457, respectively (Table 2).

DISCUSSION

Sjöholm and Laurell (1973) reported the changes of electrophoretic mobility due to conversion of C4 in serum or plasma during electrophoresis or storage. Recently, Scharfstein *et al.* (1978) described human C4-binding protein which combines the activated C4 (C4b), resulting in the greater electrophoretic mobility than the native C4. It was pointed out, therefore, that it is necessary in C4 typing to prevent conversion (or activation), and to distinguish the patterns of such conversion products from the native ones. In the present study, serum was not used as samples for this reason.

In the present study, two different electrophoretic systems were compared with each other. The agarose gel system is advantageous in its short running time. On the contrary, in the slab polyacrylamide gel system, C4 was considerably more stable and the typing was found to be easier than in the former procedure. In both methods, three common C4 phenotypes were distinguishable and the classification of the three phenotypes in the present study was considered to be identical with that reported by Teisberg *et al.* (1976, 1977) and Mauff *et al.* (1978).

On the other hand, in both electrophoretic systems in the present study, minor bands of C4F or C4S types tended to become stronger as storage periods were prolonged. Moreover, in the course of the population study, several atypical phenotypes were observed, some of which were not reproducible and the genetic basis not confirmed. So, these were considered to be due to conversion of C4. However, some other atypical phenotypes were probably rare genetic variants. Since family study could not be carried out, these were excluded from the population study.

Teisberg *et al.* (1976) and Mauff *et al.* (1978) reported the existense of two common alleles, while Teisberg *et al.* (1977) proposed the occurrence of a new common allele, $C4^{F1}$. In the present study, however, only two common alleles were confirmed.

O'Neill *et al.* (1978a, b) proposed that C4 polymorphism was controlled by two genetic loci each with a pair of dominant and recessive alleles. However, distributions of phenotypes in other reports (Teisberg *et al.*, 1976, 1977; Mauff *et al.*, 1978; present study) do not fit this model. According to this model, 'silent:recessive' alleles must be common at the C4F and C4S 'loci.' However, it has been known that C4 deficiency is extremely rare (Jersild *et al.*, 1976).

Genetic polymorphism of C4 with its high degree of heterozygosity is considered to be a particularly useful marker in human genetics, both in population and linkage studies. However, the conditions available at present for typing phenotypes seem still to be improved, since the phenotypic patterns of C4 are not as clear-cut as those in the other serum proteins, for example C3.

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