GENETIC POLYMORPHISM OF HUMAN COMPLEMENT COMPONENT C81 IN THE JAPANESE POPULATION.

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The eighth component of human complement C8 has a three chain structure composed of two subunits, α - γ and β subunits which are bound together by noncovalent bonds (Kolb and Müller-Eberhard 1976; Stechel et al.1980).

Genetic polymorphism of the C8 was first described by Raum et al.(1979), by using polyacrylamide gel isoelectric focusing (PAGIEF) of serum samples with a hemolytic assay containing a homozygous C8-deficient human serum (Petersen et al.1976) for development of patterns. Alper et al.(1983) demonstrated another C8 polymorphism which was defined by PAGIEF and development of specific patterns of hemolysis in an overlay gel containing C8 β subunit deficient serum and locus for C8 β subunit has been designated C82 with the alleles C82*A, C82*B and C82*A1. Recently, Tedesco et al. (1983) indicated that the C8-deficient serum which used for the detection of C8 polymorphism (Raum et al.1979) was characterized by the deficiency of the C8 α - γ subunit. Therefore, the locus for C8 α - γ subunit has been redesignated C81.

In the present investigation, the distribution of phenotypes and gene frequencies of C81 in the Japanese population are reported using PAGIEF followed by an electroblotting and enzymeimmunoassay with mono-specific anti C81 serum.

Materials and methods

Blood samples obtained from 448 unrelated healthy Japanese donors and 45 matings with 54 offspring were drawn onto EDTA (1.5mg/ml) as anticoagulants, and centrifuged at 2,500 rpm for 10 min to prepare the plasma.

Half millimeter thin layer polyacrylamide gel (T=5%, C= 3%) were prepared containing 3.1 M urea and 2.8 % Ampholine pH 3.5-9.5. 10 microliters of EDTA plasma were applied to the gel surface with Whatman 3 MM filter paper (5X5 mm) at a distance of 1.5 cm from the anodal end of the gel. 1.0 M H_3PO_4 (anode) and 1.0 M NaOH (cathode) were used for the electrode solution. IEF was carried out at a constant power of 10 W, maximally voltage of 1,000 V for 3 hours including the prefocusing without samples for 40 min. All experiment were conducted at 4°C.

Subsequent to the separation by IEF, transfer of protein onto a nitrocellulose membrane (0.45 μm), as previously described by Nakamura et al.(1984), was carried out for 50 min in the electrode buffer consisting 25 mM Tris-192 mM glycine and 20 % methanol, pH 8.3 at 400 mA with a cooling temperature of 4°C. For the identification of C81 pheno-

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types, the rabbit mono-specific anti human C81 serum which were a gift of Dr. K. Yamamoto, Kanazawa University School of Medicine and a peroxidase conjugated anti rabbit immunoglobulin.

Results and discussion

Phenotypes of C81 detected in the Japanese population were classified into three common and four rare patterns, and these were considered to be controlled by two common alleles, C81*A and C81*B, and three rare alleles which were tentatively designated C81*A1J, C81*A2 for acidic variants and C81*B1 for basic variant. So each typical type were designated to be C81A, C81AB, C81B, C81A1JA, C81A1JB, C81A2A and C81B1B, respectively. In this study, 3.1 M urea was added in the PAGIEF gels in order to obtain the clear-cut bands of C81 phenotypes (Alper et al.1983). C81A1JA and C81A1JB are similar to C81A and C81B, respectively, but these types have the same bands in the more anodal region than C81A. C81A2A has three major bands and one anodal and cathodal minor band, and C81B1B has C81B components and also some more cathodal bands than C81B and its major band corresponded in position to the minor cathodal band of C81B. C81 A2A and C81B1B were new rare variants detected in the Japanese population. Although C81A1JA and C81A1JB which were expressed by two common alleles and C81*A1J allele were also rare variants detected in the Japanese population, but these variants might correspond to C81A1A and C81A1B in the previous report (Raum et al.1979; Rittner et al.1984). Because the major cathodal band of C81A1J component roughly corresponded in position to the minor anodal band of C81A in the same manner as C81A1. Neuraminidase treated EDTA plasma samples from the same individuals with typical types were subjected to PAGIEF followed by an electroblotting with a mono-specific anti C81 serum, and a schematic diagram of these desialylized band patterns are shown in figure 1. But only major bands of each typical type were presented in this figure.

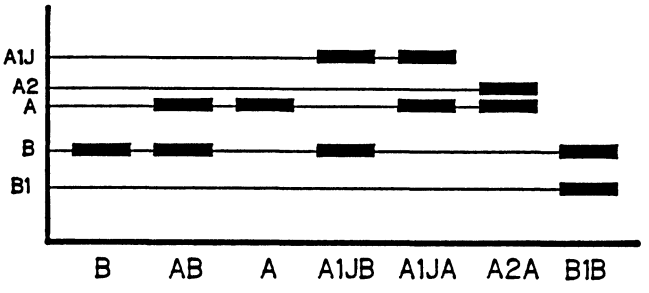

Figure 1. Schematic diagram of C81 phenotypes using PAGIEF of neuraminidase treated EDTA plasma samples followed by an electroblotting technique.

The treatment of EDTA plasma samples with neuraminidase reduced the heterogeneity of C81 types to one or two major and some minor components, and made it easy and clear to classify these types. Especially, it was easy to differentiate C81A1J component from C81A or C81B by using our method with neuraminidase treated plasma samples.

Family studies including 45 matings with 54 offspring were done to test the genetic hypothesis. It was confirmed that the C81 alleles were controlled by autosomal codominant Mendelian inheritance consisting of a single locus. No evidence of a null allele was found by studying these 45 families, and unexpected types were not observed in the children.

Table 1. Distribution of phenotypes and gene frequencies of human C81 among unrelated Japanese blood donors.

Phenotypes	Observed no.	Percent	Expected no.	x^2
A	174	38.8	173.77	0.000
AB	205	45.8	204.91	0.000
B	60	13.4	60.41	0.003
A1JA	4	0.9	4.35	0.029
A1JB	3	0.7	2.57	0.073
A2A	1	0.2	0.61	0.243
B1B	1	0.2	0.36	1.125
Others	0	0.0	1.02	1.022
Total	448	100.0	448.00	2.495

C81*A =0.6228 C81*A2=0.0011 0.5<P<0.7
C81*B =0.3672 C81*B1=0.0011 (df=4)
C81*A1J=0.0078

Distribution of phenotypes and gene frequencies of C81 in the Japanese population are presented in Table 1. The gene frequencies were estimated to be 0.6228, 0.3672, 0.0078 0.0011 and 0.0011 for C81*A, C81*B, C81*A1J, C81*A2 and C81*B1, respectively. The distribution of phenotypes fitted the Hardy-Weinberg equilibrium. The C81*A was the most common allele in the Japanese population, and the gene frequencies of two common alleles, C81*A and C81*B, agreed approximately with other ethnic groups.

A single exclusion chance for putative fathers in paternity cases was calculated as 19.3 %, so it was indicated that the C81 was the useful genetic marker in paternity test.

The presented data suggest the applicability of C81 polymorphism in forensic science, human genetics and anthropologic studies.

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