Genetic polymorphism of human complement component C81 in the Japanese population

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Summary. Genetic polymorphism of human C81 has been investigated using polyacrylamide gel isoelectric focusing (PAGIEF) in the presence of 3.1 M urea followed by electroblotting with enzyme immunoassay. In 448 individuals phenotypes of C81 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 and C81*A2J for acidic variants and C81*B1J for the basic variant. The alleles of C81*A2J and C81*B1J are new rare alleles, but C81*A1J might correspond to C81*A1 in the former studies. Family data were in accordance with the hereditary rules. The gene frequencies were estimated as C81*A is 0.6228, C81*B is 0.3672, C81*A1J is 0.0078, C81*A2J is 0.0011, and C81*B1J is 0.0011, respectively. The gene frequencies of the two common alleles agreed approximately with other ethnic groups. PAGIEF of neuraminidase-treated plasma samples followed by electroblotting with enzyme immunoassay is applicable to the study of heterogeneity of C81.

Introduction

Genetic polymorphism of the eighth component of human complement, C8, was first described by Raum et al. (1979), by using polyacrylamide gel isoelectric focusing of serum samples with a hemolytic assay containing a homozygous C8-deficient human serum (Petersen et al. 1976) for development of patterns. It has been suggested that two common alleles, C8*A and C8*B, were identified in the three racial groups, furthermore, a third common allele C8*A1 was found in blacks and whites, and these alleles were inherited in a normal autosomal codominant fashions.

It is known from previous reports that C8 has a three-chain structure composed of two subunits, α-γ and β subunits which are bound together by noncovalent bonds (Koib and Müller-Eberhard 1976; Steckel et al. 1980). Recently, Tedesco et al. (1983) demonstrated that the inherited deficiency of C8 fell into two groups. The first group was missing the β chain and the second group was missing the α-γ chain of the C8 molecule. Furthermore, they also indicated that the C8-deficient serum which was used for the detection of C8 polymorphism (Raum et al. 1979) was characterized by the deficiency of the C8 α-γ chain. Therefore, the locus for C8 α-γ chain has been redesignated C81. Rogde et al. (1985) demonstrated using two-dimensional electrophoresis that the C81 polymorphism resides in the structural gene of the C8 α chain. In 1983, Alper et al. demonstrated another C8 polymorphism which was defined by isoelectric focusing of serum in polyacrylamide gel and development of specific patterns of hemolysis in an overlay gel containing C8 β-chain deficient serum. The locus for C8 β-chain has been designated C82 with the alleles C82*A, C82*B, and C82*A1. Rittner et al. (1984) have previously described phenotypes and C81 alleles and their frequencies in the German population, and Rogde et al. (1985) have also reported both C81 (α-γ) and C82 (β) polymorphisms in the Norwegian population from the same gel with isoelectric focusing followed by immunoblotting. In the present investigation, the distribution of phenotypes and gene frequencies of C81 (α-γ) in the Japanese population is reported using polyacrylamide gel isoelectric focusing followed by electrophoretic blotting and enzyme immunoassay.

Materials and methods

Blood samples obtained from 448 unrelated healthy Japanese donors and 47 matings with 57 offspring were drawn onto EDTA (1.5 mg/ml) as anticoagulant, and centrifuged at 2500 rpm for 10 min to prepare the plasma. Plasma was stored at -80°C until use. Neuraminidase treatment of EDTA plasma samples was carried out as previously described (Nakamura and Abe 1985).

Isoelectric focusing electrophoresis (IEF)

Half millimeter thin layer polyacrylamide gels (T=5%, C=3%) were prepared containing 3.1 M urea and 2.8% (w/v) Ampholine pH 3.5–9.5 (1818-101, LKB, Sweden). IEF was carried out as follows. Ten microliters of EDTA plasma or neuraminidase treated plasma were applied to the gel surface with Whatman 3MM filter paper (5 × 5 mm) at a distance of 1.5 cm from the anodal end of the gel; 1.0 M H3PO4 (anode) and 1.0 M NaOH (cathode) were used for the electrode solution. IEF was carried out at a constant power of 10 W, maximum voltage of 1,000 V for 3 h with Ultrophor system.
Fig. 1. Electrophoretic band patterns of C81 phenotypes using PAGIEF of EDTA plasma samples followed by electroblotting and enzyme immunoassay with monospecific anti C81 serum. Anode is at top. Phenotypes from left to right: A (I), AB (II), B (III), A2JA (IV), B, A1JA (V), A1JB (VI), AB, B1JB (VII), A1JB, A, A1JB, A, A1JA, B, and B

Fig. 2. Schematic diagram of C81 phenotypes using PAGIEF of EDTA plasma samples followed by electroblotting and enzyme immunoassay with monospecific anti-C81 serum. Anode is at top. Phenotypes from left to right: B, AB, A, A1JB, A1JA, A2JA, and B1JB

Results and discussion

Electrophoretic band patterns

Figure 1 presents the C81 patterns obtained from 448 Japanese plasma samples using PAGIEF followed by electroblotting with a monospecific anti-C81 serum. Figure 2 is a schematic diagram of these band patterns. In this study, 3.1 M urea was added to the PAGIEF gels to obtain the clear-cut bands of C81 (Alper et al. 1983). In these samples three different common patterns I, II, and III, and four rare variant patterns IV, V, VI, and VII were observed. Rogde et al. (1985) reported that two distinct regions of immunologically detectable C8 bands were observed using isoelectric focusing of serum followed by immunoblotting with anti-C8 serum obtained commercially. They were called A (acidic) which represented the α-γ complex and B (basic).

Phenotyping of C81 was also examined with anti-C8 sera obtained commercially from Miles Laboratories and Atlantic Antibodies, but IEF band patterns in 3.1 M urea gels were the same as in Fig. 1 and band patterns in the B region which represented complete C8 molecules had disappeared. Type I has two major bands and one minor cathodal band, type III has two major bands and one minor band in an anodal and a cathodal position, respectively, and type II has a composite pattern of I and III. These three different common patterns were considered to be C81A, C81B, and C81AB, respectively, using the nomenclature proposed by Alper et al. (1983). Type IV has three major bands and one anodal minor band and one cathodal minor band; types V and VI are similar to C81A and C81B, respectively, but these types have the same bands in a more anodal region than C81A. Type VII has