Polymorphism of the transferrin in Japanese monkeys detected by two-dimensional gel electrophoresis

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INTRODUCTION

Japanese monkeys (Macaca fuscata fuscata) are characterized by the lower estimates of the genetic variability than the standard level observed in some other animal species. Nozawa et al.1) analyzed the 32 genetic loci for a total of 1646 blood samples from the 33 troops of Japanese monkeys. The genetic variability in individual troops quantified by the proportion of polymorphic loci and the average heterozygosity per individual were estimated to be 9.2% and 1.3%, respectively. As for the polymorphism of transferrin (Tf) of Japanese monkeys, 5 components, F, G, G-, E and H-, have been analyzed by starch gel electrophoresis1-4). The gene frequency of TfF allele is extremely high. The occurrence of the phenotypes except two, Tf FF and Tf FG, are rare. In the present study, we focused on analysis of the transferrin components of Japanese monkeys using the methods of two-dimensional gel electrophoresis (2DE) for the detection of the polymorphism in Tf subcomponents.

MATERIALS AND METHODS

Venous serum samples were collected from 16 Japanese monkeys captured in Nara Prefecture and kept at Nagano Chausuyama Zoo and stored at -20°C until use. Two-dimensional gel electrophoresis in the absence of denaturing agent was performed essentially as described by Manabe et al.5) with a following modification: Ampholine mixture of 2% pH 5-7 and 0.5% pH 3.5-9.5 were used in the first dimensional disk gel. The immunofixation was performed by anti-human transferrin after electrophoresis. Blotting procedure and immunological detection was performed essentially according to the method of Towbin et al.6). Anti-human transferrin rabbit serum as the first antibody and peroxidase conjugated goat anti-rabbit immunoglobulin as the second antibody were used. For the detection of transferrin phenotypes starch gel electrophoresis with discontinuous system of buffer was carried out according to the method of Poulik7).
RESULTS AND DISCUSSION

The variant spots with approximate pI Values of 5-6 were observed in the serum proteins of Japanese monkeys by two-dimensional gel electrophoresis. These spots were detected by immunofixation (Fig. 1) or by Western blotting and immunological detection with anti-human Tf (not shown). Two Tf phenotypes were observed in this study, which might be Tf FF and Tf FG, when these samples were examined by starch gel electrophoresis (Fig. 2). The samples from Tf FF phenotype revealed three spots and those from Tf FG phenotype had extra three spots which were more anodal and faster in the second dimensional electrophoresis (Fig. 3). Among 16 Japanese monkeys, 13 of them were Tf FF type and 3 were Tf FG type. The

Fig. 1. (A) Two-dimensional gel electrophoretic patterns of Japanese monkeys. (B) Patterns of transferrin phenotypes of Japanese monkeys detected by immunofixation using anti-human Tf followed by two-dimensional gel electrophoresis.
Left: Tf FF  Right: Tf FG

Fig. 2. Transferrin patterns of Japanese monkeys after starch gel electrophoresis.

Fig. 3. Transferrin patterns of Japanese monkeys analyzed by two-dimensional gel electrophoresis. (A) is electrophoretic patterns of Tf FF and (B) is that of Tf FG samples.
modification of the patterns by neuraminidase treatment suggested that Tf F and Tf G subcomponents contained neuraminic acid. However, the fact that Tf polymorphism was still present in the sera even after the treatment with neuraminidase suggested that Tf polymorphism was not caused by neuraminic acid but due to difference in the primary structures. In human, Tf phenotypes except Tf C detected by starch gel electrophoresis were extremely rare8,9). However, Tf C were divided into two subtypes, Tf C1 and Tf C2 by isoelectric focusing electrophoresis10). Thereby, a greater extent of Tf polymorphic variation was revealed. No polymorphism of Tf subcomponents in Japanese monkeys were found in this study. Further analysis of the Tf in Japanese monkeys by the methods of two-dimensional gel electrophoresis should be informative for genetic study.

REFERENCES