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Paternity Discrimination in a Japanese Macaque Group by DNA Fingerprinting

MIHO INOUE, AKIKO TAKENAKA, SHOJI TANAKA,
RYO KOMINAMI, and OSAMU TAKENAKA
Kyoto University

ABSTRACT. Recently developed DNA fingerprinting techniques employing "minisatellite" hypervariable regions of DNA proved useful for investigating male reproductive success in Japanese macaques (*Macaca fuscata*), for which other conventional behavioral or biochemical methods were impracticable. The identified paternity in a captive group indicated that inbreeding was avoided within the same maternal lineage and that females did not tend to give birth to offspring fathered by the same males during their life. It also revealed the possibility of a correlation between male dominance rank and number of offspring.

Key Words: Paternity discrimination; Japanese macaques; Minisatellite DNA; Social rank; Inbreeding avoidance.

INTRODUCTION

Paternity in Japanese macaque groups has been behaviorally indiscernible because of their multimale group structure and promiscuous mating patterns. Electrophoretic analysis of blood proteins and immunoserological analysis have also failed to discriminate paternity because there is little detectable polymorphism. HAYASAKA et al. (1986) pointed out that paternity exclusion requires the electrophoretic analysis of several hundred genetic loci.

This situation contrasts with that in other macaques, such as *M. fascicularis* (SHIVELY & SMITH, 1985) and *M. mulatta* (SMITH, 1981, 1982a, b; CURIE-COHEN et al., 1983; STERN & SMITH, 1984; SMITH & SMITH, 1988), which have more polymorphism in their blood proteins as detected by electrophoretic analysis. The above reports, for artificially formed groups, achieved 70–80% paternity exclusion and dealt with the male social status and their reproductive success. Some authors concluded that there were no significant relationships between the males' social rank and the number of their offspring (SHIVELY & SMITH, 1985; CURIE-COHEN et al., 1983), whereas another concluded that reproductive success of the adult males was statistically significantly correlated with rank (SMITH, 1981).

Paternity discrimination in Japanese macaques requires novel genetic markers. Recently, JEFFREYS and others have explored the type of hypervariable regions of DNA called "minisatellites" (WYMAN & WHITE, 1980; JEFFREYS, WILSON, & THEIN, 1985a, b; JEFFREYS, BROOKFIELD, & SEMEONOFF, 1985; GILL et al., 1985; WONG et al., 1986; JEFFREYS et al., 1986; NAKAMURA et al., 1987; VASSART et al., 1987; HILL, 1987; WETTON et al., 1987; JEFFREYS et al., 1987; JEFFREYS & MORTON, 1987; KOMINAMI et al., 1988; JEFFREYS et al., 1988; WEISS et al., 1988). Molecular probes for tandem-repetitive DNA in humans have also been found to be useful for paternity identification in mice (JEFFREYS et al., 1987), dogs (JEFFREYS & MORTON, 1987), cats (JEFFREYS & MORTON, 1987), and even birds (HILL, 1987; WETTON et al., 1987).

The present report describes the results of paternity discrimination in a Japanese macaque group employing hypervariable genetic markers on DNA generated by synthesized minisatellite probes.

MATERIALS AND METHODS

MATERIALS

A wild Japanese macaque group was introduced to the Primate Research Institute, Kyoto University, from Tottori Prefecture, western Japan, in 1974, and named the Wakasa group. Two males and eight females were selected to make up the group which was kept in an open enclosure of 500 m^2 in area. In 1988, the group consisted of 19 males and 30 females with 8 infants of different ages. The group produced 48 offspring during the 14 years. Six males were removed from the group. Two males (*Nos. 1* and *2*) and five of the females (*Nos. 247*, *251*, *252*, *255*, and *260*) remained members from the beginning. Each individual was identified by tatooing, and the complete maternal family tree is available.

DISCRIMINATION PROCEDURES

High molecular DNA (200–300 μg) was prepared from 10 ml of peripheral blood of the macaques. Four microgram of each DNA sample was digested with restriction enzyme, HinfI or HaeIII. The digests were precipitated with ethanol and separated by electrophoresis through a 20-cm-long 0.7% agarose gel at 45 V for 22 hr. The DNA was denatured and transferred to a nylon membrane filter (Hybond-N). The filter was hybridized with ^{32}P-labelled single stranded minisatellite probes 33.15 (JEFFREYS, WILSON, & THEIN, 1985a), myo, and core (KOMINAMI et al., 1988). The consensus sequences of the 33.15, myo, and core were 5′-AGAGGTGGGCAGGTGG-3′, 5′-GACCGAGGTCTAAAGCTGGAGGTGGGCAGGAAG-3′, and 5′-GGAGGTGGGCAGGAGG-3′. Southern blot hybridization and washing were performed in 3×SSC at 65°C. The filter was autoradiographed at −80°C for four to ten days. Resultant band patterns are shown in Figure 1. The probe corresponding to JEFFREYS' probe 33.15 was prepared as follows. Oligonucleotide, tandem duplicate of the 16 core sequence, and its complementary nucleotide were synthesized using a DNA synthesizer (Applied Biosystems, 381A). These nucleotides were slipped off respectively by four bases so that the double strand had cohesive 5′ ends consisting of four bases. After ligation, the nucleotide fraction over 180 bp was blunted with Klenow fragment and ligated into the SmaI site of the plasmid vector, pUC 18. The host strain was *E. coli* JM103.

RESULTS AND DISCUSSION

MALE DOMINANCE RANK AND NUMBER OF OFFSPRING

Figure 2 shows the number of offspring and their fathers in each year. For several years after the establishment of the group, only *No. 1* male sired offspring. Later, however, various males joined in the breeding of the group. *No. 3* male, born in 1976, impregnated females in the 1981 mating season at the age of about 5.5, although male Japanese macaques generally begin to mount and ejaculate at 4.5 years. There was no change in dominance rank among the two adult males, *No. 1* and *No. 2*, in 1974–1979 and or among the three adult males,

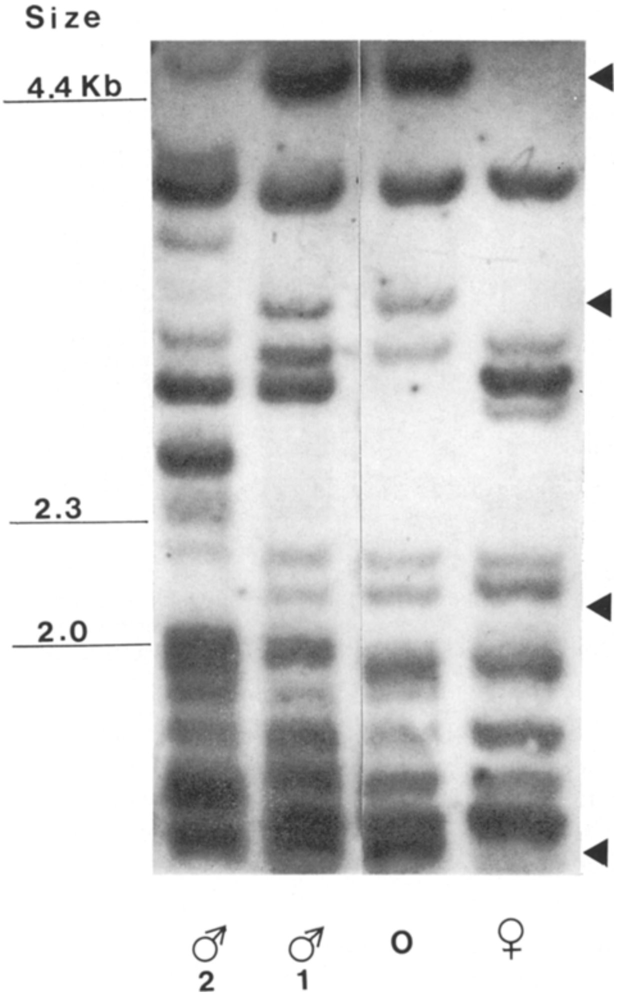

Fig. 1. Pattern of minisatellite DNA. An example of paternity discrimination by DNA fingerprinting is shown. Four bands indicated by black triangles are possessed by the offspring (O), but not possessed by its mother (♀). One of two candidates for the father, ♂1, has all four bands but ♂2 does not. This set of data indicates that these three bands possessed by the child were inherited from its father, ♂1.

Table 1. Number of offspring of males in 1988.

Name	Age	No. of offspring	%	Maximum possible No. of offspring if maternal inbreeding is avoided	%
No. 1	19	1	12.5	8	12.5
No. 2	17	2	25.0	8	25.0
No. 3	11	1	12.5	5	20.0
No. 5	6	2	25.0	8	25.0
No. 6	7	1	12.5	7	14.3
No. 7	7	0	0.0	8	0.0
No. 8	5	1	12.5	8	12.5
No. 9	5	0	0.0	8	0.0
No. 10	5	0	0.0	5	0.0
Total		8	100.0		

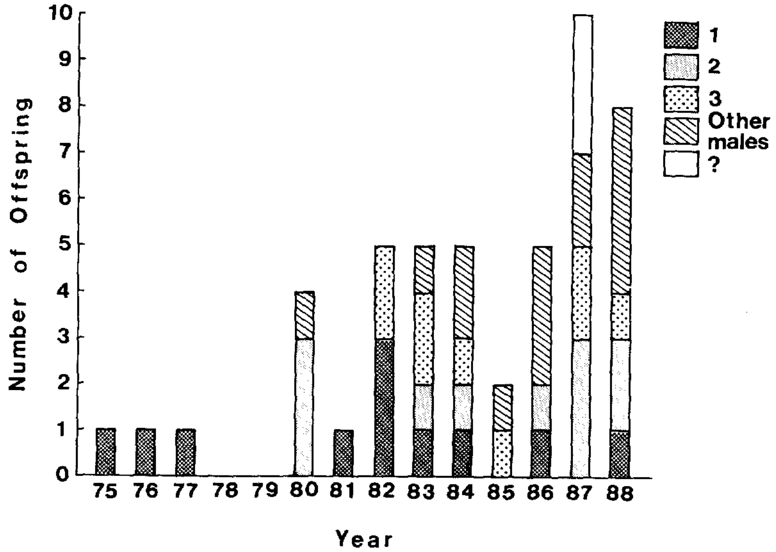

Fig. 2. Reproductive success of males in the Japanese macaque group studied. The numbers of offspring of males in each year are shown. Bars with tight stippling, fine stippling, and open stippling indicate the offspring of the *No. 1*, *No. 2*, and *No. 3* males, respectively. Bars with oblique shading represent offspring of other males. Open bars indicate offspring whose father could not be identified.

No. 1, *No. 2*, and *No. 3*, in 1980–1988. It is often speculated from observations of mating behavior that the higher the dominance rank of a male the more offspring he can sire. In total, 41 offspring were born in the period after 1981 when all of the three males had reached sexual maturity. In each of the years from 1981 to 1988, the number of offspring of the three males did not always reflect their rank order.

Table 2. Binominal tests of inbreeding avoidance.

	Possible No. of offspring	No. of offspring	P	n	r	$\sum_{i=r}^{n} {}_nC_i\, P^i(1-P)^{n-i}$
Matrilineal family						
No. 3			16/41	9	0	*0.01*<0.05
Out of the family	25	9				
In the family	16	0				
Total	41	9				
Patrilineal family						
No. 1			11/48	11	1	0.95>*0.24*>0.05
Out of the family	37	10				
In the family	11	1				
Total	48	11				
No. 2			6/48	11	1	0.95>*0.59*>0.05
Out of the family	42	10				
In the family	6	1				
Total	48	11				
No. 3			10/41	9	3	0.95>*0.85*>0.05
Out of the family	31	6				
In the family	10	3				
Total	41	9				

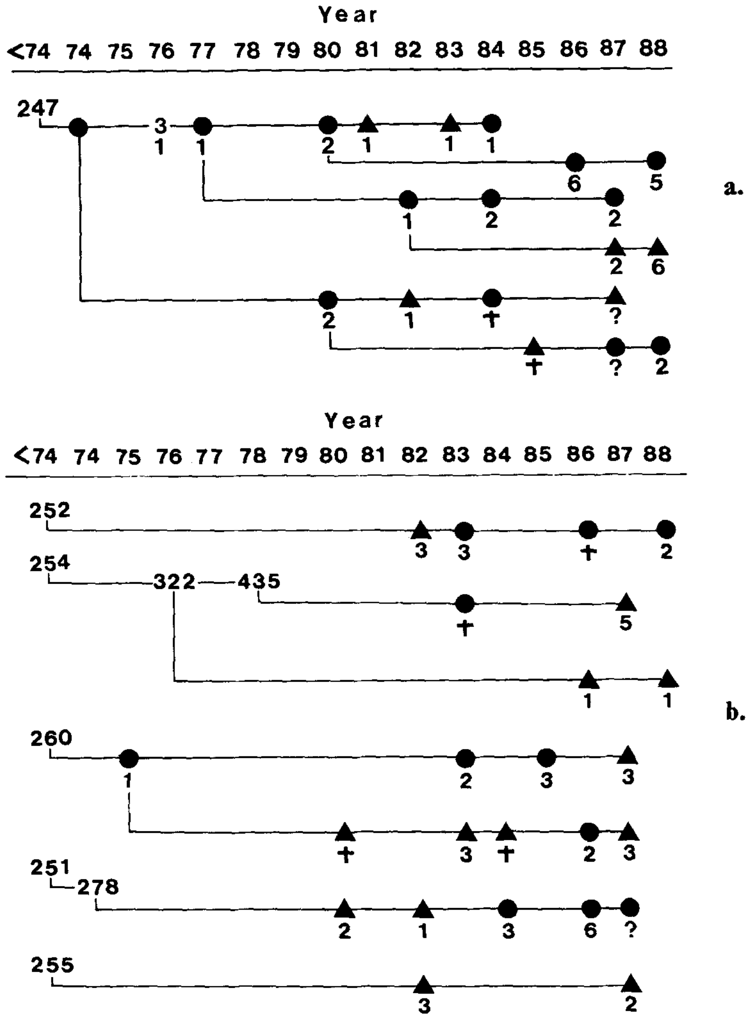

Fig. 3. Maternal lineages in the study group and the offspring's fathers. Circles represent female offspring and triangles male. The number below each mark is the individual number of the father. In the case of a cross (+), the father was considered to be one of the dead males. The paternity of the three question-marked individuals could not been identified. **a.** Maternal lineage of female *No. 247*. The numeral 3 instead of a triangle signifies *No. 3* born in 1976. He fathered none of the 16 offspring of this family. For the two question-marked offspring, *No. 3* male was ruled out. **b.** Maternal lineages of females *No. 252*, *254*, *260*, *251*, and *255*. *No. 254* has died, and the fathers of *Nos. 322* and *435* were not identified.

Table 1 summarizes the number of offspring of nine males aged ≥ 5 years in 1988. Both of the two 4-year-old males had no offspring. A weak correlation existed between the male dominance rank and the number of offspring ($r_s = 0.70$, $0.01 < p < 0.05$). On the other hand, there was no significant correlation between male age and the number of offspring ($r_s = 0.45$, $p > 0.05$). More data on the lifetime reproductive success of males are needed to reach a more definitive conclusion.

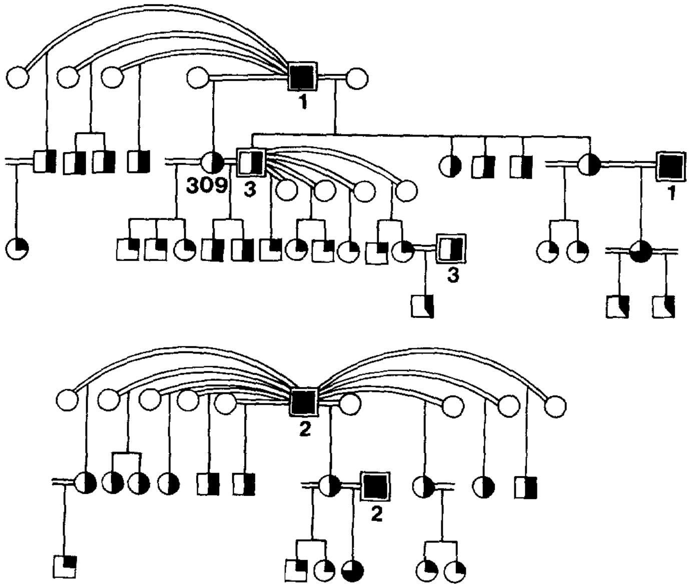

Fig. 4. Paternal lineages in the study group. The upper part of the figure depicts the lineage of the *No. 1* and *No. 3* males and the lower part is that of *No. 2*. The lineages for only these three males are shown since 65% of the offspring in this group were theirs. Circles represent females and squares males. The black part of each mark represents the proportion of genes inherited from *No. 1* (upper) or *No. 2* (lower).

INBREEDING AVOIDANCE AMONG MATERNAL LINEAGE

Table 2 summarizes the results of binominal tests of inbreeding avoidance. In the studied group, which has kept in an enclosure, males could not emigrate from their natal group. In spite of such circumstances, inbreeding was avoided in the same maternal lineage. Figure 3a shows the lineage of the female named *No. 247*. *No. 3* male was a son of *No. 247*. Among the 41 offspring born since 1981, when *No. 3* become sexually mature, 16 were his matrilineal family members and 25 were members of other families. He sired no offspring in his own family and nine in the other families. The number of offspring of *No. 3* in his family was significantly low (binominal test, $p<0.05$). This suggests that inbreeding was avoided in the same matrilineal family, although incestuous copulations among maternal kin were observed.

In contrast, inbreeding was not avoided in father-daughter relationships and between paternal half siblings. For example, *No. 247* gave birth to a daughter in 1977 whose father was *No. 1*. The daughter gave birth to a grandchild of *No. 247* in 1982, whose father was again *No. 1*. As seen in Figure 4, *No. 3* male and *No. 309* female were half siblings, and they had two offspring between them. *No. 1* had 48 possible offspring in 1975–1988 (Fig. 2). There were 11 possible patrilineal incestuous offspring, as seen in Figure 4, and 37 possible non-incestuous children. He sired one incestuous offspring and ten non-incestuous offspring. The number of incestuous offspring of *No. 1* was not significantly lower than expected. For *No.* 2 and *No. 3*, the number was not significantly lower than expected.

In the wild, Japanese macaque males emigrate from their natal group at between 3 and 6 years old (Sugiyama, 1976). Although this phenomenon has been regarded as one of the socio-ecological mechanisms for inbreeding avoidance, the present study indicated that 5-year-old males possibly sire their offspring in their natal group. These results imply that monkeys may discriminate their maternal relatives, probably on the basis of their matrilineal group structure, while they do not discriminate paternal relatives.

FEMALES DID NOT TEND TO BREED THEIR OFFSPRING BETWEEN THE SAME MALES DURING THEIR LIFE

Figures 3a and 3b show maternal family trees of females who gave birth to two or more offspring. Except for one female, *No. 322*, females gave birth to offspring of $\geqq 2$ males. Thus, female Japanese macaques do not tend to breed their offspring between the same males year by year, indicating there are few mating pairs which are stable over the years among them.

In the present study, we were able to discriminate the paternity of 45 out of 48 offspring. For the three non-discriminated individuals, we could not exclude all of the non-father males because they had some bands in common. New probes are needed to identify the fathers of these three individuals.

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REFERENCES

CURIE-COHEN, M., D. YOSHIHARA, L. LUTTRELL, K. BENFORADO, J. MCCLUER, & W. H. STONE, 1983. The effects of dominance on mating behavior and paternity in a captive troop of rhesus monkeys (*Macaca mulatta*). *Amer. J. Primatol.*, 5: 127–138.

GILL, P., A. J. JEFFREYS, & D. J. WERRETT, 1985. Forensic application of DNA 'fingerprints.' *Nature*, 318: 577–579.

HAYASAKA, K., Y. KAWAMOTO, T. SHOTAKE, & K. NOZAWA, 1986. Probability of paternity exclusion and the number of loci needed to determine the fathers in a troop of macaques. *Primates*, 27: 103–114.

HILL, W. G., 1987. DNA fingerprints applied to animal and bird populations. *Nature*, 327: 98–99.

JEFFREYS, A. J., J. F. Y. BROOKFIELD, & R. SEMEONOFF, 1985. Positive identification of an immigration test-case using human DNA fingerprints. *Nature*, 317: 818–819.

——— & D. B. MORTON, 1987. DNA fingerprinting of dogs and cats. *Anim. Genet.*, 18: 1–15.

———, N. J. ROYLE, V. WILSON, & Z. WONG, 1988. Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. *Nature*, 332: 278–281.

———, V. WILSON, R. KELLY, B. A. TAYLOR, & G. BULFIELD, 1987. Mouse DNA 'fingerprints': Analysis of chromosome localization and germ-line stability of hypervariable loci in recombinant inbred strains. *Nucleic Acids Res.*, 15: 2823–2826.

———, ———, & S. L. THEIN, 1985a. Hypervariable 'minisatellite' regions in human DNA. *Nature*, 314: 67–73.

———, ———, & ———, 1985b. Individual-specific 'fingerprints' of human DNA. *Nature*, 316: 76–79.

———, ———, ———, D. J. WEATHERALL, & B. A. J. PONDER, 1986. DNA "fingerprints" and segregation analysis of multiple markers in human pedigrees. *Amer. J. Human Genet.*, 39: 11–24.

KOMINAMI, R., K. MITANI, & M. MURAMATSU, 1988. Nucleotide sequence of a mouse minisatellite DNA. *Nucleic Acids Res.*, 16: 1197.

NAKAMURA, Y., M. LEPPERT, P. O'CONELL, R. WOLFF, T. HOLM, M. CULVER, C. MARTIN, E. FUJIMOTO, M. HOFF, E. KUMULIN, & R. WHITE, 1987. Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science*, 235: 1616–1622.

SHIVELY, C. & D. G. SMITH, 1985. Social status and reproductive success of male *Macaca fascicularis*. *Amer. J. Primatol.*, 9: 129–135.

SMITH, D. G., 1981. The association between rank and reproductive success of male rhesus monkeys. *Amer. J. Primatol.*, 1: 83–90.

————, 1982a. Inbreeding in three captive groups of rhesus monkeys. *Amer. J. Phys. Anthropol.*, 58: 447–451.

————, 1982b. A test of randomness of paternity of members of maternal sibships in six captive groups of rhesus monkeys (*Macaca mulatta*). *Int. J. Primatol.*, 3: 461–468.

———— & S. SMITH, 1988. Paternal rank and reproductive success of natal rhesus males. *Anim. Behav.*, 36: 554–562.

STERN, B. R. & D. G. SMITH, 1984. Sexual behavior and paternity in three captive groups of rhesus monkeys (*Macaca mulatta*). *Anim. Behav.*, 32: 23–32.

SUGIYAMA, Y., 1976. Life history of male Japanese monkeys. In: *Advances in the Study of Behavior, Vol. 7*, J. S. ROSENBLATT, R. A. HINDE, E. SHAW, & C. BEER (eds.), Academic Press, Inc., New York, pp. 255–284.

VASSART, G., M. GEORGES, R. MONSIEUR, H. BROCAS, A. S. LEQUARRE, & D. CHRISTOPHE, 1987. A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. *Science*, 235: 683–684.

WEISS, M. L., V. WILSON, C. CHAN, T. TURNER, & A. J. JEFFREYS, 1988. Application of DNA fingerprinting probes to Old World monkeys. *Amer. J. Primatol.*, 16: 73–79.

WETTON, J. H., R. E. CARTER, D. T. PARKIN, & D. WALTERS, 1987. Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature*, 327: 147–152.

WONG, Z., V. WILSON, A. J. JEFFREYS, & S. L. THEIN, 1986. Cloning a selected fragment from a human DNA 'fingerprint': Isolation of an extremely polymorphic minisatellite. *Nucleic Acids Res.*, 14: 4605–4615.

WYMAN, A. R. & R. WHITE, 1980. A highly polymorphic locus in human DNA. *Genetics*, 77: 6754–6758.

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Authors' Names and Addresses: MIHO INOUE and AKIKO TAKENAKA, *Department of Biochemistry, Primate Research Institute, Kyoto University, Inuyama, Aichi, 484 Japan*; SHOJI TANAKA, *Suntory Institute for Biomedical Research, Mishima-gun, Osaka, 618 Japan*; RYO KOMINAMI, *Faculty of Medicine, Niigata University, Niigata, 951 Japan*; OSAMU TAKENAKA, *Department of Biochemistry, Primate Research Institute, Kyoto University, Inuyama, Aichi, 484 Japan.*