

Sequence register

Sequence analysis of *DPB1*-like genes in cynomolgus monkeys (*Macaca fascicularis*)

Katsuko Hashiba¹, Shoji Kuwata², Katsushi Tokunaga³, Takeo Juji², ³, Atsuo Noguchi¹

- ¹ Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki-ken 305, Japan
- ² Department of Transfusion Medicine and Immunohematology, Faculty of Medicine, University of Tokyo, 7 Hongo, Bunkyo-ku, Tokyo 113, Japan
- ³ Department of Research, Japanese Red Cross Central Blood Center, 4-1-31 Hiroo, Shibuya-ku, Tokyo 150, Japan

Received April 6, 1993/Revised version received June 1, 1993

Two sequences of *Mafa-DPB1*-like alleles of the cynomolgus monkey major histocompatibility complex were determined without using cloning techniques. In the present study, eight cynomolgus monkeys belonging to two families (Fig. 1) were selected from the Malaysian colonies at the Tsukuba Primate Center. Using the polymerase chain reaction (PCR)-SSCP method (Hoshino et al. 1992) with a primer set (Kimura and Sasazuki 1992) for the human DPB1 exon 2, two different homozygous patterns as well as several heterozygous patterns were identified (Fig. 1A, B). Subsequently, the two sequences of the homozygous DNAs were determined using an automated DNA sequencer. The nucleotide sequences of these two alleles (Mafa-DPB1*M25 and M09; Fig. 2) were closer to those of human DPB1 alleles (the average percentage identity between the nucleotide sequences of the two macaque alleles and those of human alleles DPB1*02011, 0401. and 0101 is 89%; Marsh and Bodmer 1991) than to the human DPB2 pseudogene (75%; Kapper and Strominger 1986). The findings obtained by comparing the two macaque allele sequences were consistent with the observation that there are many more nonsynonymous than synonymous substitutions at the human HLA-DPB1 locus. The present results clearly demonstrate that cynomolgus monkeys have DPB1-like alleles. It is presumed, therefore, that macaques have at least one DPB1 locus and that nonsynonymous nucleotide substitutions are prevalent in the gene.

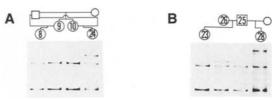


Fig. 1A, B. Leukocyte DNAs from family members indicated at the top (\bigcirc , females; \square , males) were subjected to PCR-SSCP analysis. Since the mobility shifts of each set of single-strande DNA fragments of A Nos. 8, 9, and 10 and B 23 and 25 were the same, and two bands were observed, they were determined to be homozygotes having equivalent DNA fragments.

Mafa-DPB1*N25 Mafa-DPB1*N09		AG	AAT 	TAC	CTG	TAC	10 CAG	GGA	CGG	CAG	GAA	TGC	TAC	GCG	ATT	AAC	20 GGG	ACA
Mafa-DPB1 *M25 Mafa-DPBI *M 09			TTC					ATC	30 TAC	AAC	CGG	GAG	GAG	TTC	ATG	CGC	TTC	GAC
Mafa-DPB1*M25 Mafa-DPB1*M09	€0 AGC	GAC	GTG -CC	GGG	GAG	TAC	CGG	GCG	GTG	ACA	50 GAG	ATG	GGA	C00	CCT	ACT	GCG	GAA
Mafa-DPB1*M25 Mafa-DPB1*M09	TAC	TGC G	SO AAC	AGC	CAG	AAG	GAC	AAA CGC	CTG	GAG	GAG	ATG -a-	70 CGG	GCA C	GTG -A-	GCG -T-	GAC	AGG
Mafa-DPB1*M25 Mafa-DPB1*M09	ATG G	TGC	AGA	CAC T	AAC	TAC	GAG	GTG -G-	AAC C	GAG	GCT A	GTG	ACC	CTG	90 CAG -G-	CGC	CGA	G

Fig. 2. Nucleotide sequences of exon 2 in two *Mafa-DPB1* alleles. Sequence identity is indicated by *dashes*.

References

Hoshino, S., Kimura, A., Fukuda, Y., Dohi, K., and Sasazuki, T. Polymerase chain reaction-single-strand conformation polymorphism in *DPA1* and *DPB1* genes: a simple, economical, and rapid method for histocompatibility testing. *Hum Immunol 33*: 98–107, 1992

Kapper, D. J. and Strominger, J. L. Structure and evolution of the HLA class II SX β gene. Immunogenetics 24: 1–7, 1986

Kimura, A. and Sasazuki T. Eleventh International Histocompatibility Workshop reference protocol for the HLA DNA – typing technique. In K. Tsuji, M. Aizawa, and T. Sasazuki (eds): HLA 1991, vol. 1., pp. 397–418, Oxford University Press, Oxford, 1992

Marsh, S. G. E. and Bodmer, J. G. HLA Class II nucleotide sequences, 1991. Hum Immunol 31: 207-227, 1991

The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession numbers D13335 (Mafa-DPB1*M09) and D13336 (Mafa-DPB1*M25.)