ANALYSIS OF HLA-DRB1 ALLELES USING PCR-RFLP AND PCR-MPH

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Abstract. In this study we compare the results of HLA-DRB1 genotyping by PCR-RFLP and PCR-MPH. HLA-DR specificities were also performed by LCT. Samples were obtained from 20 Thai patients who were on the waiting list for kidney transplant. DNA was extracted by phenol-chloroform extraction. It was found that the results gave complete agreement with two methods of DNA typing, However, there were 3 discrepancies in assigning serologic DR specificities and DNA subtypes ($p = 0.0001$) which were due to the cross reactive antibodies and the lack of potent antisera to define proper HLA-DR subtypes by LCT. These PCR techniques can be applied to identify other alleles such as HLA-DPB1 and HLA-DQB1 which will improve the standard histocompatibility testing in the future.

INTRODUCTION

The analysis of HLA class II alleles, especially HLA-DR, is important in tissue and bone marrow transplantation, disease susceptibility and forensic studies. HLA-DR typing was originally performed by the lymphocytotoxicity test (LCT) (Terasaki *et al*, 1978). However, it was not sensitive to resolve all HLA class II alleles. At present, the polymerase chain reaction (PCR) techniques have been studied, such as PCR-SSO (sequence-specific oligonucleotide), PCR-RFLP (restriction fragment length polymorphism), PCR-MPH (microplate hybridization) in order to get precise definition of HLA-DR alleles at the DNA level (Mizuki *et al*, 1990; Inoko, 1990; Uryo *et al*, 1990; Ota *et al* 1992; Kawai *et al*, 1993, 1994).

The PCR-RFLP method is based on digestion of PCR- amplified DNA samples with allele specific restriction endonucleases. The PCR-MPH is similar to an enzyme-linked immunosorbent assay (ELISA) in that labeled PCR products are hybridized to the immobilized probes on a microtiter well and the bound PCR products are detected colorimetrically by means of standard biotin-streptavidin method.

We performed HLA-DRB1 genotyping of Thai patients by means of these two methods and compared the results to each other to assess the technical reliability of these methods.

MATERIALS AND METHODS

DNA samples

DNA samples were obtained from 20 Thai patients who were on the waiting list for kidney transplant at Pramongkutklao Hospital, Bangkok, Thailand. DNA was extracted by phenol-chloroform extraction according to the 11th International Histocompatibility Workshop protocol. The HLA-DR specificities of all subjects were also determined by the serological LCT method (Terasaki *et al*, 1978; Milken, 1987).

PCR-RFLP

RFLP analysis was performed according to the method described by Inoko *et al* (Inoko *et al*, 1990; Uryo *et al*, 1990; Ota *et al*, 1992). Genomic DNA was amplified using 7 group specific primers : DR1, DR2, DR52 associated (DR 3, 5, 6, 8), DR3, DR7, DR9 and DR10 antigen specific primers in order to obtain the amplified product from DRB1 gene. Following amplification, the amplified products were digested with specific restriction enzymes. Samples of the restriction enzyme cleaved amplified DNA were subjected to electrophoresis in 10% polyacrylamide gel in a minigel apparatus (Mupid, Cosmo Bio Co Ltd). Restriction fragments were detected by staining with ehtidium bromide.

PCR-MPH

MPH analysis was performed according to Kawai *et al* (1994) with a few modifications. The ss-DNA containing tandemly ligated probe units free from the complementary strand was immobilized onto microtiter wells (Wakunaga Pharmaceutical Co Ltd Japan). Exon-2 of the HLA-DRB1 alleles was amplified by PCR using biotinylated primers at the 5' termini. After amplification, the denatured PCR products were then hybridized with the immobilized probes on the well. After washing, the PCR products bound to the well were detected with peroxidase-conjugated streptavidin and a chromogenic substrate. The absorbance of each well was read at 415 nm by a microtiter plate reader.

RESULTS

As seen in Table 1, it was notable that the results gave complete agreement in DRB1 typing by PCR-RFLP, PCR-MPH. However, there were some discrepancies between the serologic assignments of HLA-DR and the DNA-typing data for generic DRB1 on 3 samples ($p = 0.0001$). The first discrepancy was noted with specimen no. 42 which showed the serologic type HLA-DR 15, 11 but showed DRB1 *1501, *1405 by DNA-typing. The false serologic assignment was related to erroneous assignment of the other DR6 cross reactive antigen especially DR5.

The second discrepancy was noted with specimens no. 253 and no. 254 which showed the serologic type HLA DR4, - and HLA DR2, -; while DNA-typing information defined DRB1 *0405, *1302 and DRB1 *1602, *1404, respectively. This serological error could reflect the potency of antibody especially for DR13 and DR14 in the HLA class II typing tray.

DISCUSSION

In this study, the DNA typing : PCR-RFLP and PCR-MPH gave correlative results. On the other

Table 1

Comparison results in HLA-DRB1 typing by different techniques.

No.	DNA No.	DR SEROLOGY	DRB1* PCR-RFLP	DR PCR-MPH
1	002	2, 11	1602, 1101	2, 11
2	003	15, 12	1502, 1202	2, 12
3	004	1, 4	0101, 0405	1, 4
4	042	15, 11#	0501, 1405	2, 6
5	061	15, 4	1501, 0403	2, 4
6	154	2, 4	1602, 0403	2, 4
7	168	12, -	1202, -	12, -
8	189	15, 4	1501, 0403	2, 4
9	245	14, -	1404, -	6, -
10	242	15, 11	1502, 1101	2, 5
11	244	11, 12	110 (1, 3 = 4), 1202	11, 12
12	252	15, 12	1502, 1202	2, 12
13	247	7, 12	07, 1202	7, 12
14	246	4, 6	0405, 1401	4, 6
15	253	4, -**	0405, 1302	4, 6
16	256	15, 11	1502, 1103 = 4	2, 11
17	241	7, 14	1404, 07	6, 7
18	254	2, -**	1602, 1404	2, 6
19	255	ND	1602, 07	2, 7
20	243	ND	0401, 1202	4, 12

Serological result was wrong (no. 42)

** Serological results were DR blank but both were DR6 by the other techniques (No. 253, 254)

hand, discrepancies in assigning serologic DR specificities and DNA subtypes have been noted in 3 samples ($p = 0.0001$). This was due to the cross reactive antibodies and the lack of potent antisera to define the proper assignments of the subtypes of DR specificities by LCT. Our experience seemed similar to the report by ASHI/CAP DR survey program that DNA-typing has been helpful in clarifying the serological definition of DR and DQ subtypes (Duquesnoy *et al*, 1994).

From this study, PCR-RFLP was found to be a sensitive and reliable method for HLA DRB1 genotyping. The problem of incomplete or partial digestion of the PCR products by restriction enzymes can be overcome by preparation of positive control DNA samples for ensuring that the digestion is complete. PCR-MPH was simple as well as capable of processing large numbers of samples. This method could be replaced by conventional LCT for routine DR typing but it needs more careful monitoring for the constant temperature of hybridization and washing conditions. In order to resolve the indistinguishable results of two samples: no. 244 and no. 256, the further double digestion of PCR products with Cfr13 I and Fok I should be done.

In conclusion, we suggest that PCR-RFLP is proven to be an excellent technique for routine HLA-DRB1 typing. On the other hand, PCR-MPH can be performed to screen for HLA-DRB1 alleles when approaching with large numbers of samples. These PCR techniques can be applied to identify other alleles such as HLA-DPB1 and HLA-DQB1 and which will improve standard histocompatibility testing in the future.

ACKNOWLEDGEMENTS

This study was supported in part from the NRCT-JSPS cooperation programs (JFY 1994) and the Osaka Red Cross Blood Center in Japan. We thank Ms Kazue Konishi, Ms Yang Sook Ko and Mr Matsuyama Nobuki for their technical advice and Mr Thomas Mcmanamon for advice in the preparation of this manuscript.

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