COMPARATIVE ANALYSIS OF SEROLOGICAL AND MOLECULAR RESULTS FOR HLA-DR TYPING IN 120 THAI SUBJECTS

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Abstract. In this study, serological HLA-DR typing results were compared to typing results obtained with sequence-specific primers in the polymerase chain reaction (PCR - SSP). HLA-DR typing was performed on 120 random Thai individuals. Differences in HLA-DR typing results were found in 18 out of 120, which were due to cross reactive antibodies and the lack of potent antisera to define proper HLA-DR splits by serology. Furthermore, PCR-SSP is fast and easy to perform as HLA-DR typing results can be obtained within 2 hours. From the results of this study it can be concluded that PCR-SSP is a reliable and promising technique for HLA-DR typing which can replace the serological technique in routine clinical practice.

INTRODUCTION

Typing of human leukocyte antigens (HLA), especially HLA-DR, allows the matching of patients and donors in clinical transplantation, and HLA-matching is a major factor prolonging the life of transplanted grafts (Dyer and Claas, 1997). Serological HLA-DR typing was originally performed by microlymphocytotoxicity test (Terasaki *et al*, 1978). Subsequently, the test was modified, becoming a one-step monoclonal antibody-typing procedure in order to shorten the incubation time (Lee *et al*, 1994). However, the limitations in HLA-DR typing by serology are due to the availability of antisera reactive with certain HLA-DR types and the low quality or quantity of B lymphocytes obtained by venipuncture of patients. In order to increase the accuracy of HLA genotyping, polymerase chain reaction (PCR) technics have been developed such as PCR-RFLP (restriction fragment length polymorphism), PCR-MPH (microplate hybridization) and PCR-SSP (sequence-specific primers) (Inoko, 1990; Uryo *et al*, 1990; Ota *et al*, 1992; Olerup and Zetterquist, 1992; Kawai *et al*, 1994).

The PCR-SSP method is based on the principle that a completely matched primer will be more efficiently used in the PCR reaction than a primer with one or several mismatches, especially in the first critical cycles. Thus, the specificity of the typing system is part of the PCR reaction. Assignment of alleles is based on the presence or absence of amplified product, which can be detected by agarose gel electrophoresis. The rapid and simple post-amplification processing of samples makes this technique very practical for tissue typing in clinical practice (Zetterquist *et al*, 1997). The purpose of this study was to evaluate the benefits achieved by PCR-SSP HLA-DR typing compared to conventional serological typing.

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MATERIALS AND METHODS

Subjects

One hundred and twenty random Thai individual blood samples were collected and analyzed by serological HLA-DR typing using HLA class II typing trays DR 72 F and PCR-SSP HLA-DR typing using micro SSP™ Generic HLA Class II DNA typing kits (One Lambda, Inc, USA). To validate the test results, PCR-RFLP was carried out using group-specific-DRB1 primers similar to the protocol described previously (Ota *et al*, 1992).

Serology

Thirty milliliters of ACD blood for each sample was typed by the microlymphocytotoxicity test using a one-step monoclonal antibody typing tray (Lee *et al*, 1994). HLA-DR serology was performed on B cells isolated from bulk lymphocytes (ficoll gradients) by nylon adherance. All reactions were examined under the inverted phase contrast microscope and scored by estimating the percentage of cell deaths beyond that of the background or negative control as recommended by the ASHI standard

scoring system (Milken, 1987). The following 14 DR specificities were recognized: DR1, 15, 16, 17, 18, 4, 11, 12, 13, 14, 7, 8, 9 and 10.

PCR-SSP

DNA was extracted directly from 2 ml of EDTA blood using the salting out method. Each DNA sample (100 ng/μl) was tested using the micro SSP DRB/DQB generic kit (One Lambda, Inc, USA). Briefly, the DNA sample (100 ng) was amplified with 31 different primer sets which had been optimized and dispensed into each well of a thin-walled 96 well PCR plate for HLA class II low resolution typing. The SSP-DNA reaction set was placed in the PTC 200 thermal cycler (MJ Research, Inc, USA). The cycle parameters of the PCR program began with a first step of 1 cycle of 130 seconds at 94°C and 60 seconds at 63°C, followed by 9 cycles of 10 seconds at 94°C, 60 seconds at 63°C and finally, 20 cycles of 10 seconds at 94°C, 50 seconds at 59°C and 30 seconds at 72°C. The last step was to hold the sample at 4°C. After amplification, 10 μl of each PCR reaction was transferred in sequence to a 2.5% agarose gel with 0.5 μg/ml ethidium bromide and electrophoresed at 150 volts for 4 minutes. The reaction pattern was photographed and the assessment of HLA alleles was performed by analysing of the gel banding pattern using a reaction pattern typing grid.

RESULTS

HLA-DR typing results obtained from 120 Thai individuals were compared between serology and PCR-SSP. The results showed 85% (102/120) concordance between the two methods. It was found that a discrepancy in results had been noted in 18 individuals (Table1). First, there was a difference between the conclusive heterozygous assignments by the two technics in samples no. 6, 36, 50, 81 and 101. Second, there was an inconclusive assignment by serology which generally occured when the percentage of B cells killed was in between the values of the background and the positive control in samples no. 42 and 43. Third, there was a missed antigen/allele, which was defined by the absence of appropriate HLA-DR antigen/allele assignment by serological technic in samples no. 3, 18, 59, 71, 79, 97 and 98. Finally, the false positive DR11 in sample no.46 and DR13 in samples no.20, 92 and 106 were found only by serology, not by PCR-SSP. These results were also confirmed by PCR-RFLP.

DISCUSSION

A reliable HLA typing method is considered to be imperative for suitable kidney or bone marrow transplantation. Although HLA-DR typing by serological techniques are still performed, the resolution, accuracy and reproducibility of serological HLA-DR typing have been shown to be inferior to PCR-SSP or PCR-RFLP HLA-DR typing (Olerup and Zetterquist, 1992; Nathalang *et al*, 1996; Zetterquist *et al*, 1997). In the present study, HLA-DR typing results showed 85% concordance between serology and PCR-SSP. The discrepancies were due to the cross reactive antibodies and the lack of potent antisera to define the proper assignments of the splits of DR specificities especially DR 2, 5 and 6. Furthermore, technical failures and inconclusive HLA-DR serological assignments were found in 2 samples which were due to the low quantity or quality of B cells in patients' blood samples that might not satisfy minimal criteria needed for successful typing. Sufficient DNA could be extracted and HLA-DR typing by PCR-SSP could be performed. An additional advantage of PCR-SSP is that it is less time-consuming and HLA-DR typing results are obtained in less than 2 hours (Olerup and Zetterquist, 1992; Otten *et al*, 1995).

Table 1
Comparison of the differences HLA-DR typing results generated by serology and PCR-SSP.

No.	Sample no.	DR serology	DRB1* PCR-SSP
1	003	15,-	15,16
2	006	8,14	12,14
3	018	15,-	15,16
4	020	15,13	15,15
5	036	16,4	15, 4
6	042	- ,-*	01,12
7	043	- ,-*	15,07
8	046	15,11	15,15
9	050	2,4	15,04
10	059	14,-	13,14
11	071	3,-	03,14
12	079	11,-	11,13
13	081	15,-	16,16
14	092	15,13	15,15
15	097	10,-	10,12
16	098	10,-	10,03
17	101	2,4	15, 4
18	106	12,13	12,12

* Only weak reactions were obtained by serological method.

In conclusion, unlike serology, PCR-SSP is a reliable method for HLA-DR typing. In addition, this method is fast, easy to perform and the results are not difficult to interpret. Therefore, in the near future HLA-DR typing by serology will be replaced by PCR-SSP in routine clinical practice.

REFERENCES

Dyer PA, Claas FHJ. A future for HLA matching in clinical transplantation. *Eur J Immunogenet* 1997; 24: 17-28.

Inoko H. PCR-RFLP method holds great promise for complete HLA class II genotyping. *Tissue Antigens* 1990; 36: 88-92.

Kawai S, Maekawajiri S, Tokunaga K, *et al.* A simple method of HLA-DRB typing using enzymatically amplified DNA and immobilized probes on microtiter plate. *Human Immunol* 1994; 41: 121-6.

Lee J-H, Lias M, Deng C-T, *et al.* A one step monoclonal antibody typing procedure that simplifies HLA class I and class II typing. *Tissue Antigens* 1994; 44: 34-42.

Milken SL. Reagent antisera for HLA typing. Tissue typing reference manual. South-Eastern Organ Procurement Foundation, 2nd ed. 1987; 1-11.

Na Thalang O, Sukha C, Suwanasophon C, *et al.* Analysis of HLA-DRB1 alleles using PCR-RFLP and PCR-MPH. *Southeast Asian J Trop Med Public Health* 1997; 27: 169-71.

Olerup O, Zetterquist H. HLA-DR by PCR amplification with sequence specific primers (PCR-SSP) in 2 hours : an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens* 1992; 39: 225-35.

Ota M, Seki T, Fukushima K, *et al.* HLA-DRB1 genotyping by modified PCR-RFLP method combined with group-specific primers. *Tissue Antigens* 1992; 39: 187-202.

Otten HG, Tilanus MGJ, Barnstijn M, *et al.* Serology versus PCR-SSP in typing for HLA-DR and HLA-DQ : a practical evaluation. *Tissue Antigens* 1995; 45: 36-40.

Terasaki PI, Bernoco D, Park MS, *et al.* Microdroplet testing for HLA-A, -B, -C and -D antigens. *Am J Clin Pathol* 1978; 69: 109-20.

Uryo N, Maeda M, Ota M, *et al.* A simple and rapid method for HLA-DRB and -DQB typing by digestion of PCR-amplified DNA with allele specific restriction endonucleases. *Tissue Antigens* 1990; 35: 20-31.

Zetterquist H, Bengtsson M, Backstrom G, *et al.* Report from the HLA class II typing by PCR-SSP multicentre study. *Eur J Immunogenet* 1997; 24: 191-9.