Comparison between serological and molecular typing for HLA Class II antigens (HLA-DR and DQ)

Dhaliwal JS, Maiselamah Lisut, Lee Yin Yin and Nasuruddin BA *Division of Immunology, Institute for Medical Research, 50588 Kuala Lumpur*

Abstract

A total of 17 subjects were typed for HLA-DR Class II antigens using one serologic and two DNA typing kits. Our results showed that DNA typing is superior to serologic typing in assigning the correct HLA type. A total of 8 incorrect assignments and 6 missing assignments were noted. Seven HLA-DQ alleles and 1 HLA-DR8 allele were incorrectly assigned. Discrepancies due to a failure in identifying DR8 (2 cases), DQ6 (2 cases) DR 52 (1 case) and DR 51 (1 case) were also observed.

Key words: HLA; DNA; PCR

Introduction

The human major histocompatability complex (MHC) refers to a cluster of genes located on the short arm of chromosome 6. These genes encode the Human Leukocyte Antigen (HLA) Class I (HLA-A, B, C), Class II (HLA-DR, DQ, DP) and complement (BF, C2, C4A and C4B) molecules. Class I antigens are expressed on the surface of most nucleated cells while Class II antigens have a more restricted distribution being expressed largely on B cells, activated T cells and antigen presenting cells. These molecules play a crucial role in antigen presentation as well as being involved in organ rejection and graft versus host disease. In order to increase the success rate of transplantation, Class I and Class II alleles are typed to select the best recipient-donor match.

Serologic methods have slowly given way to molecular techniques. This has simplified the identification of HLA antigens because the differences are identified directly at the DNA level. Initial efforts to use DNA technology for HLA typing were largely restricted to Class II loci which have a smaller degree of polymorphism compared to Class I loci. Serologically, it is technically more difficult to assign Class II antigens because of the paucity of mono-specific antisera. In addition, Class II antigens, unlike Class I antigens have a restricted distribution which requires the isolation of B cells. Currently, at least five DNA based methods have been reported for HLA typing. These are (1) the hybridisation of PCR products with sequence specific oligonucleotide probes (PCR-SSOP) (Saiki *et al.*, 1986), (2) Restriction Fragment Length Polymorphism (RFLP) on PCR amplified product (Carrson *et al.*, 1987), (3) Single Stranded Conformation Polymorphism (SSCP) (Kimura et al., 1992), (4) PCR-SSP or PCR amplification with sequence specific primer (Olerup & Zetterquist, 1992) and (5) Direct sequencing of PCR amplified product (Santamaria et al., 1992). A number of laboratories have adopted these methods not only for Class II but also for Class I antigens. In this paper, we describe the establishment, in our laboratory, of PCR-SSP typing for Class II antigens using two different commercial kits and compare it with the serological method currently used.

Materials and Methods

Subjects

At the Division of Immunology, Institute for Medical Research, Kuala Lumpur, Class I and Class II HLA typing is carried out to support the Bone Marrow Transplant Program. Patient or donor cells left over from 20 ml of blood sent for serological typing were used to carry out HLA DNA typing. A total of 18 specimens were tested using three different kits.

Serologic typing

Typing was carried out using serologic kits (Lambda Monoclonal HLA Class II Tissue Typing Trays obtained from I-Lambda Inc Canoga Park, CA) (Kit A). Briefly, white blood cells were separated from 20 ml of peripheral blood using Lymphoprep followed by B cell isolation using Dynal beads (Dynabeads, Dynal AS, Norway). The test was carried out according to the manufacturer's recommendations. Lymphocytotoxicity was evaluated with a fluorescent microscope using acridine orange to determine the percentage of cell death.

DNA extraction

DNA was extracted from buffy coats or Lymphoprep isolated white blood cells sent for HLA typing to our laboratory. DNA extraction was carried out using QIAamp Blood Kit (Qiagen Corp. CA) or High Pure PCR Template Preparation

kit (Boehringer Mannheim GmbH). Buffy coats from approximately 2 ml of blood or T cells from 15 ml of blood were re-suspended in distilled water and DNA extracted according to the manufacturer's recommendations. The concentration and purity of the extracted DNA was determined using a Turner 690 UV spectrophotometer. The DNA obtained ranged, in concentration, from 6ng/µl to 216 ng/µl. The total DNA used was adjusted to fall between 750 - 1,500 ng for Kit B and 975-7,800 ng DNA for Kit C.

PCR kits

Micro SSP Generic HLA Class II kits DR and DQ SSP Typing from Biosynthesis Inc, (Lewisville TX, USA) (Kit B) and One Lambda Inc (Canoga Park, CA) (Kit C) were used for HLA DNA typing. The cycling conditions for Kit C were: 94°C for 130 s, 63°C for 60 s, Cycle: 94°C for 10 s, 63°C for 60 s, repeat for 9 cycles, Cycle: 94°C for 10 s, 59°C for 50 s, 72°C for 30 s, repeat for 20 cycles. The cycling conditions for Kit B were as follows: 96°C for 60 s, cycle : 94°C for 20 s, 65°C for 60 s, repeat for 10 cycles, cycle: 94°C for 20 s, 61°C for 50 s, 72°C for 30 s. Repeat for 20 cycles followed by 5 minutes at 72°C. Taq polymerase was purchased from Boehringher Manheim GmbH. The PCR products were visualised after electrophoresis in 2% Agarose gel and staining for 30 minutes with Ethidium bromide. The reaction patterns were interpreted according to the chart provided by the manufacturer.

Results

Commercial serologic and DNA kits were used to type eighteen patients and donors for Class II (DR and DQ) antigens. The results obtained (Table 1) from these kits are largely comparable. However, discrepant results were noted. These could be classified as alleles mistakenly assigned as blanks or results that were not concordant with the assignments obtained using other kits. A total of 8 incorrect assignments and 6 missing assignments were noted. Seven HLA-DQ and 1 HLA-DR alleles were incorrectly identified. Discrepancies due to a failure in identifying DR8 (2 cases), DQ6 (2 cases) DR 52 (1 case) and DR 51 (1 case) were also seen. The main source of error found in Kit A was the difficulty in assigning alleles to resolve DQ5 and DQ6. The reason for this is that assignments were done based on the strength of the reaction of 2 monoclonals. However, if the subjects were DQ5, DQ6, then Kit A would be unable to determine this. Discrepancies must be studied carefully to determine if any error has occurred. Inserts included with the kit usually detail the limitations in detecting certain allelic combinations. Ambiguous results were reconfirmed using high resolution kits from 1-Lambda Inc.

Discussion

In our laboratory, Class I and Class II HLA typing is carried out using the microlymphocytotoxicity assay which was first described by Terasaki and McClealland in 1964. This is a complement dependent assay using panels of selected HLA alloantisera. However, this method has a number of drawbacks. The primary disadvantage is that it is highly dependent on the purity, viability and quality of B cells. This may be difficult to control especially if the patient is in relapse. In our case, it is sometimes further compounded by the distances the blood has to be transported over. There is also a paucity of mono-specific antisera. These factors, however, are of minor consequence when using molecular techniques. In addition to accuracy, there are a number of qualitative advantages in using molecular methods for the identification of HLA antigens. Only small volumes of blood are required. This is not only useful when dealing with paediatric cases but is essential for specialised needs, for example the setting up of a cord blood regisry. Poli *et al.* (1998) has shown that standard microcytotoxocity testing for Class I antigens was unsatisfactory for 14.5 % of the 1,644 cord blood samples that were typed. Furthermore, specimens can be re-tested without recalling the patient and a permanent record of the results may be kept. The most critical factor in determining the success or failure of molecular techniques is the quantity and quality of the DNA.

The discrepancies shown in Table 1 may be broadly classified as antigens incorrectly assigned as blanks or results that were not concordant with the serologic assignments. This failure to identify the presence of an allele is a major problem in serologic typing (Lau *et al.*, 1994). A high error rate in the serologic definition of HLA-B molecules has been documented (Lorentz *et al.*, 1997; Bozon *et al.*, 1997). Such errors result from a misinterpretation of reaction patterns of clusters of antisera most of which contain more than one antibody specificity. In general, there was concordance for results obtained using molecular techniques although the level of resolution between kits was not always comparable. This, however, only occasionally poses problems in our case as we deal largely with related donors. In cases of matched-unrelated donor BMT DNA typing,

Table 1. Results of PCR-SSP and the serologic typing for HLA Class II (DR & DQ) antigens

Name	Loci	*Results	Kit A (Serologic)	Kit B (PCR-SSP)	Kit C (PCR-SSP)	Comments
ET	DR	3,4,52.53	**No result	3,4,52,53	3,4,52,53	
	DQ	2,4		2,4	2,4	
AA	DR	12,15,51,52	**No result	12,15,51,52	12,15,51,52	
	DQ	3.6		3.6	3,6	
JM	DR	4,15,51,53	4,15,51,53	4,15,51,53	4,15,51,53	
	DQ	5	6	5	5	[a]Incorrect
LYJ	DR	9,12,52,53	n.d.	9,12,52,53	9,12,52.53	
	DQ	3		3	3	
NF	DR	15,51	15,51	15,51	15,51	
	DQ	5	6	5	5	[a]Incorrect
MM	DR	4,15,51,53	4,15,53	4,15,51,53	4,15,51,53	[b]Missing
	DQ	5	6	5	5	[a]Incorrect
CCK	DR	8,14,52	8,14	8,14,52	8,14.52	[b]Missing
	DQ	5,6	5	5,6	5,6	[e]Missing
CTK	DR	8,14,52	14,52	8,14,52	8,14,52	[d]Missing
	DQ	5,6	5	5,6	5,6	[c]Missing
TCL	DR	8,9,53	9,53	8,9,53	8,9,53	[d]Missing
	DQ	3	3	3	3	
JD	DR	4,15,51,53	4,15,51,53	4,15,51,53	4,15,51,53	
	DQ	5	6	5	5	[a]Incorrect
FD	DR	15.51	15.51	15,51	15,51	
	DQ	5	6	5	5	[a]Incorrect
NR	DR	15,51	15,51	15,51	15.51	
	DQ	5	6	5	5	[a]Incorrect
SM	DR	14,52	8,14	14,52	14,52	[e]Incorrect
	DQ	5	6	5	5	[a]Incorrect
A	DR	9,53	9,53	9,53	9,53	
	DQ	3	9 (3)	3	3	
MHK	DR	9,53	9,53	9,53	9.53	
	DQ	3	9 (3)	3	3	
T6	DR	9,15,51,53	n.d.**	9,15,51.53	9,15,51,53	
	DQ	3,5		3,5	3,5	
T7	DR	8,12,52	n.d.**	8,12,52	8,12,52	
	DQ	3,5		3,5	3,5	

*Results were obtained by considering all 3 kits and by carrying out high resolution typing where necessary; **Cell mortality was in excess of 30%; [a]The serologic kit is dependent on the subjective determination of strength of the reaction; [b]DR15 is closely associated with DR51; [c]Kit A cannot distinguish if both DR5 and DR6 are positive; [d]The presence of DR8 was not detected; [e]The presence of DR 8 was incorrectly detected

DNA typing would be considerably better than serological typing.

On the whole DNA typing techniques are superior to serologic typing. It is important to establish that the accuracy of the results is maintained over a period of time when dealing with a large number of specimens. Cost is sometimes cited as an obstacle. However, if repeats, blanks and incomplete data were taken into account the difference in cost is not large. Each laboratory should decide on the combination of typing methods to be utilised based on the type of patients, the cost and the screening protocol adopted.

Acknowledgements

The authors would like to thank Mr Ong Kian Joo and the staff of the Tissue Typing Laboratory, Division of Immunology, for their assistance and the Director, Institute for Medical Research for permission to publish this paper.

References

Bozon MV, Delgado JC, Selvakumar A, Clavijo OP, Salazar M, Ohashi M, Alosco SM, Russell J, Yu N, Dupont B & Yunis EJ (1997). Error rate for HLA-B antigen assignment by serology: implications for proficiency testing and utilizaion of DNAbased typing methods. *Tissue Antigens* **50**, 387-394.

Carsson B, Wallin J, Boheme J & Moller E (1987). HLA DR-DQ haplotypes defined by restriction fragment length polymorphism analysis : correlation to serology. *Human Immunology* **20**, 95-113.

Kimura A, Hoshino S, Yoshida M, Harada H & Sasasuki T (1992). Polymerase chain reation -single strand conformation polymorphism (PCR-SSCP) analysis of HLA genes : A practcal HLA-matching system. In : Tstiji K, Aizawa M, Sasazuki T, eds *HLA 1991*, Oxford : Oxford University Press, 1992, pp. 511-514.

Lau M, Terasaki PI & Park MS (1994). International Cell Exchange 1994 : In: *Clinical Transplants* 1993. Los Angeles UCLA Tissue Typing Labs (1994), pp. 467-488.

Lorentz DF, Iwanaga, KK, Meuer KJ, Moritz TL & Watkins DI (1997). A 25% error rate in the tying of HLA-B homozygotes. *Tissue Antigens* **50**, 359-365.

Olerup O & Zetterquist H (1992). HLA-DR typing by PCR amplification with sequence specific primers (SSPPCR) in 2 hours : an alternate to serology DR typing in clinical practice incluing donor-recipient match in cadaveric transplation. *Tissue Antigens* 1992 **39**, 225-235.

Poli F, Scalamogna M, Crespiatico L, Macchi B, Misto R, Nocco A, Rossini G, Scarpino C, Sioli V & Sirchia G (1998). Comparison of serological and molecular typing for HLA-A and -B on cord blood lymphocytes. *Tissue Antigens* **51**, 67-71.

Santamaria P, Boyce-Jacino MT, Lindstrom AL, Barbossa JJ, Faras AJ & Rich SS (1992). HLA Class II "typing" : direct sequencing of DRB, DQB and DQA genes. *Human Immunology* **33**, 69-81.

Saiki RK, Bugawan TL, Horn GT, Mullis KB & Erlish HA (1986). Analysis of enzymaticaly amplified beta-globin and HLA-DQ alpha DNA with allele specific oligonucleotide probes. *Nature* **324**, 163.

Terasaki PI & McClelland JP (1964) Microdroplet assay of human cytokines. *Nature* **204**, 998-1000.

Received 29 July 1998; accepted for publication 2 August 1998.