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HLA Typing – A Comparison of Serology and DNA Techniques

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ABSTRACT DNA based HLA typing is fast replacing conventional Microlymphocytotoxicity based method, which has been regarded as the gold standard. Many laboratories in Mumbai have already switched over to molecular methods, as the results are far superior. This study was undertaken to compare the results of generic molecular typing by sequence specific primers (SSP) with that by serology in our laboratory. A pilot study was performed in which available data tissue typing results of 200 patients and their donors were analysed. DNA was extracted from whole blood or buffy coat using QIA amp@ DNA Mini kit from Qiagen (Germany). SSP based low resolution typing was performed for 50 individuals using commercial kits from Biotest (Germany) and Genovision (USA). Microlymphocytotoxicity based tissue typing was also done for twenty individuals using commercial sera, while the remaining were typed by SSP alone. Samples were run in two different time phases labeled in Table as first and second run. DNA quality and quantity was found to be sufficient by the method for tissue typing. Of the pilot study of 200 cases typed earlier by serology, all six HLA antigens were identified only in 40% individuals, with maximum number of blanks in DR typing. DNA typing results were best for class II typing and not very satisfactory for HLA B typing. The results of DR SSP typing were far superior and almost 90% of alleles were identified. The cost of molecular typing was approximately $ 82 – 124 per sample while for serology it was $ 64 – 69. The SSP based HLA typing is an economical, rapid, precise, technically simple and reproducible method. Further the non availability of specific HLA antisera from native populations, large number of blank alleles, and comparable cost of immunomagnetic isolation of B cells, it is suggested that DNA based methods must completely replace serology.

INTRODUCTION

Serology, proliferation or cytotoxicity assays and DNA based methods have been utilized to identify HLA alleles by using living cells and serology has been considered as "Gold standard" and recognizes immunologically relevant antigenic differences (Hurley et al. 1997). In contrast DNA based testing may also identify differences of little biologic relevance. The first kidney transplant was performed in INHA Asvini on 11 Feb 1991 and since then over 200 renal transplants has been carried out till date. Till recently the tissue typing and cross matches for our recipient - donor pairs have been performed in commercial laboratories outside the hospital. On observing the records, it was observed that very often all six alleles viz. A, B and DR were not determined by Microlymphocytotoxicity (Serology) – much more often than could be explained by homozygosity or null alleles. In view of the changing donors trends seen as an increase in number of unrelated renal transplants, possible solutions are recommended to increase the frequency with which all six alleles can be determined. The relative economics of the two methods are also outlined.

MATERIALS AND METHODS

Data pertaining to a total of 200 individuals (donors and recipients) were obtained from the Department of Urology for whom the HLA typing had been done by Serology for class I and II HLA antigens. Donor patterns over the last 10 years were analysed. In INHS Asvini Sequence Specific Primers (SSP) based low resolution typing was performed for 50 individuals using commercial kits from Biotest (Germany) and Genovision (USA). Additional six tests were performed for standardization, correlation of results of two kits and standardization of the PCR thermal cycler. These six tests have been excluded from study. Taq polymerase (Fermentas or Gibco BRL) was found to be suitable and used for SSP amplification. Microlymphocytotoxicity based tissue typing was also done for twenty individuals using commercial sera, while the remaining thirty were typed by SSP alone. DNA extraction was carried out from whole blood collected in Ethylene Diamine Tetra Acetic Acid (EDTA) Vacutainers using QIA amp@ DNA Mini kit from Qiagen (Germany) and the protocol given was followed. The purified DNA was eluted with water or AE Buffer containing EDTA. DNA

quantification was done by measuring the absorbance at 260 nanometers (nm) and the purity assessed at 280 nm. The quality and quantity of the DNA was checked by gel analysis in 0.8% agarose. A crisp band close to the well correlated with a good yield of DNA. After getting 15 samples of DNA of good quality only gel analysis was carried out prior to amplification. DNA based typing was done by SSP and the thermal cycler was programmed as mentioned in the literature of kits used. After amplification the positive bands were identified by running a gel in 2% agarose. Each well had a positive control by way of a band of human growth hormone of 1069 base pairs. For samples amplified using Genovision kits the results were analysed by software whereas for Biotest kit analysis of results was done manually using a chart provided. The relative cost of HLA typing by serology and DNA (SSP) were compared.

RESULTS

The results of 200 samples carried out outside INHS Asvini by serology are shown in Table 1. The results of SSP based DNA typing for 50 cases and serology based typing for 20 cases is shown in Table 2. It is obvious that the results of HLA typing for HLA – A and DR correlated very well for Microlymphocytotoxicity and molecular typing and in nearly 84 – 90% both alleles could be identified. The results of HLA – B typing in the first run were much inferior and in the first run of 20 samples both allele were identified in only eleven cases (55%). The cost analysis of the two methods and of SSP kits provided by different vendors was carried out and the results are tabulated in Table 3. The cost per ABDR typing ranged from $82 to $124 and is still higher if licensed kits are used. Donor patterns have changed considerably over the last five years. Now wife is the commonest donor specially if the patient is her husband and female donors by far outnumber males. This has been observed after the passing of Organ Transplant act by government of India on 4th Feb, 1995. In the first fifty transplants carried out at INHS Asvini, the wife of the patient donated in 3 cases (6%), where as in 33 transplants carried out in 1998 – 2000 wife was the donor in 11 cases (33%). Another trend observed has been an increase in unrelated donors from 10% to 30%.

Table 1: Results of serology based A, B and DR typing of 200 cases

Number of patients (n)	*A*	*B*	*DR*
1 Allele determined	74 (37%)	92 (46%)	16 (58%)
2 Allele determined	126 (63%)	108 (54%)	84 (42%)

DISCUSSION

DNA based typing focuses on defining differences in genes and may identify differences of little biologic relevance whereas immune based testing by serology or cellular reactions is related to known immunological differences. Serologically DR alleles were the least frequently determined with both alleles identified in only 42% cases and in 90% cases by SSP (Table 1 and Table 2). However, this cannot be attributed to lack of expertise but to technical flaws such as poor isolation of B cells, the typing tray used. The laboratories used nylon wool for T and B cell isolation. B cells isolated by this method are very often contaminated with T cells due to which the reactions may be weak or not take place at all. Most commercial typing sera are obtained from

Table 2: Depicting results of HLA typing by serology(s) and molecular methods (m)

		First run (n = 20)			Second run (n = 30)	
Alleles	*One allele*	*Two alleles*	*Private alleles (DNA typing)*	*Difference between two %*	*One allele (%)*	*Two alleles*
A	6(s); 3(m)	14(s); 17(m)	6	8	5(10)	25(84)
B	10(s); 9(m)	10(s); 11(m)	2	20	13(43)	17(57)
DR	8(s); 2(m)	12(s);18(m)	4	5	3(10)	27(90)

Table 3: The relative cost of serology and SSP per sample

Reagent	*Serology x (SSP)*	*Molecular (SSP)*
Dry Trays	$60	—-
Immunogenetic Beads	$25	—-
Lymphoprep	$4	—-
SSP Tray	—-	$70 to $100
Taq Polymerase	—-	$8 to $20
DNA Extraction	—-	$4
Total Cost	$64 to $89	$82 to $ 124

Caucasian population and some alleles peculiar to the India population may not be identified. One experiment revealed that the purity of B cells isolated by nylon wool when tested by flowcytometry was only 55%. The possible solutions are B cell separation using a magnet and monoclonal antibodies, which costs almost $25 per isolation. Though more expensive the B cell yield is far better and the B cells can be used for both DR typing by CDC as well as for B cell cross matches. B cell cross matches have an important bearing on the outcome of a graft (Horsburgh et al. 1997) and thus should not be ignored. Another solution is to do DR typing by SSP as is being done at many labs. The bands with HLA – A and DR typing in all cases were as expected but for HLA – B typing some of the expected bands were not present. The commercial cost of generic SSP molecular typing is almost twice that of serological but as seen from above the Class II alleles may be undetermined in upto 40% cases. If immunomagnetic isolation of B cells is done and "Dry Trays" with pre dropped sera is used the cost is only marginally lower. Now SSP trays are available commercially and HLA typing can be done for both Class I and Class II alleles within two and a half hours and the results are excellent in terms of reproducibility and precision. Over 95% of the kidney transplants in India are carried out from living donor. Earlier when the donor was a sibling, parent or child it could be assumed that the allele which was not determined could be common. Secondly if the donor was a parent or child haploidentity was ensured. With the changing donor trends – viz an increase in number of spousal and other biologically unrelated transplants it is imperative to determine all six alleles correctly. There are two schools of thought among HLA specialists; one that feels only zero mismatches is of relevance. The other group subscribes to the fact that the renal graft outcome depends on the degree of mismatch and DR typing is of greatest relevance (Chritians et al. 1999). The difference between serologically and molecular typing results for Class II alleles may be upto 25% for the same patients (Bunce et al. 1997) and hence the requirement for DNA based methods. Availability of SSP kits for tissue typing with advantages at a cost similar to serology based dry kits and changing donor pattern have made it imperative for most commercial labs to switch over to DNA based methods.

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