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A single center evaluation of the Collaborative Transplant Study (CTS) DNA project

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Abstract Historical HLA class II serological typing results of transplantations performed in "The Leuven Collaborative Group for Transplantation" were subjected to retrospective Restriction Fragment Length Polymorfism (RFLP) DNA control typing by the Collaborative Transplant Study (CTS) DNA project using Polymerase Chain Reaction (PCR)-based DNA methods. We re-evaluated the serology/RFLP-discrepant CTS DNA data for our local patients transplanted during a historical period (January 1988 until May 1992) before any class II DNA typing was performed in our tissue typing laboratory. These retyping results confirm both the CTS data for patient typing and the Eurotransplant data for donor typing. A confirmed high discrepancy rate of 19.0 % (after exclusion of 2.2 % transcription errors) was found in the patient population. A low discrepancy rate of 6.8 % (after exclusion of 2.2 % transcription errors) for the donor population is concordant with the Eurotransplant donor data. Only 4 of the 588 individuals were found to be incorrectly typed by the RFLP method; all involving the specificities DRB1*1102. This indicates that RFLP typing, as performed by the CTS DNA project, can be considered a valid, retrospective DNA typing system for the accurate interpretation of class II matching in organ transplantation. A second conclusion to be drawn from this study is the need for prospective DNA typing for kidney transplant recipients, as the discrepancy rate in this cohort is high. Our results suggest that with good quality serological HLA-DR typing, prospective donor DNA typing is not urgently needed.

Key words Kidney transplantation, RFLP, DNA · Tissue typing, RFLP, kidney transplantation · RFLP, kidney transplantation · HLA matching, RFLP

Introduction

Allocation of kidneys for cadaveric renal transplantation within the Eurotransplant area is based on HLA class I and class II serological matching of at least 1 HLA-B and 1 HLA-DR antigen in combination with a negative lymphocytotoxic crossmatch. Several studies have demonstrated a better kidney graft survival when kidney recipients were given HLA-matched organs [2, 6, 14, 20]. It is known that HLA-DR serology typing is problematic and that typing errors are considerable [9]. This is mainly due to the absence of monospecific antisera and extensive crossreactions occurring between different alleles of the class II loci. With the advent of molecular biology techniques, DNA typing became available for class II tissue typing, although until recently these techniques were not fast enough for prospective donor typing. RFLP [1] was the first technique available. This technique has been used extensively by the Collaborative Transplant Study (CTS), which maintains

a worldwide registry of organ transplants. A major aim of the CTS registry is to study variables influencing kidney graft survival. In this context, much attention has been paid to the effect of HLA matching. It is generally accepted that DNA typing is more accurate for class II typing than serology.

In the CTS DNA project [2, 9, 20], donor-recipient pairs from more than 100 transplant centers were retyped for HLA Class II DR and DQ at the DNA level, and the DNA typing results were used for the evaluation of graft survival. A high discrepancy rate (25 % for donors, 27.9 % for recipients) between serology and DNA typing was found [10]. A recent study comparing Eurotransplant donor retyping results, however, showed a very low incidence (3 %) of HLA-DR "broad" discrepancies between Polymerase Chain Reaction-Sequence specific oligonucleotide hybridisation (PCR-SSO) typing and HLA-DR serology of cadaveric organ donors that were retyped in the Eurotransplant reference laboratory [23]. This frequency of incorrect HLA-DR typing results is much lower than that reported by the CTS study, where donors and recipients from all continents were tested [10].

In the present study, we re-evaluated the RFLP DNA typing results of the CTS study for our local donors and recipients (The Leuven Collaborative Group for Transplantation) [22] by applying another DNA typing technique, PCR-SSO, in order to compare the results obtained with the two DNA typing techniques and to evaluate the DNA matching effect in a single center.

Materials and methods

Materials

Patient and donor samples

Collection of cell material from kidney donors and recipients was initiated in January 1988. Transplantations performed from January 1988 to May 1992 were included in this study. A total of 300 out of 345 transplantations (86 %) were performed before 1991. For 64 donors, both kidneys were kept for transplantation in local patients. At the time of transplantation, all donors and recipients were exclusively typed by serology. Peripheral blood anticoagulated with EDTA from all recipients and a piece of spleen from all donors was collected on the day of transplantation and stored at –20 °C. One part of the stored material was sent to the CTS laboratory in Heidelberg, where DNA was isolated and RFLP typing for DRB, DQB, and DQA was performed [1, 2]. The other part was kept in our laboratory for later testing.

The RFLP results were reported by CTS to the individual participating transplant centers. Upon receipt of the RFLP results in Leuven, they were compared with the serological typing results obtained earlier in our local HLA laboratory. If an HLA-DR discrepancy was found between serology and RFLP (excluding transciption errors), a one-step PCR-SSO (low resolution) was performed for confirmation. If a discrepancy was found between RFLP and PCR-SSO, a third DNA method was used (PCR-SSP [11] and/or INNOLiPA [3]). The results of all confirmation tests are discussed together with their impact on the matching degree in our local transplant center.

HLA-DR Serology

For the period relevant for this investigation, only "broad" HLA-DR specificities (DR1-DR10) were transmitted from our center to the CTS registry. For that reason, only broad-antigen discrepancies were considered for evaluation. The recipient's HLA-DR typing was, in all cases, performed serologically on two different occasions using the two-color fluorescence lymphocytotoxicity test [15]. The first typing was performed at the time of first admission, and a second control typing on some different occasion. Two different serology trays were used. One tray was a local class II tray; the second was the reference Eurotransplant tray. In case of doubt or suspected homozygosity, family typings were performed. Donor class II serology was routinely performed on spleen cells using the same technique as for recipients and on the same local class II serum set and on the standard serum set distributed by the Eurotransplant reference laboratory [19].

Methods

RFLP procedure

DNA was extracted from peripheral blood using a salting-out method from spleen tissue as described by Graham [4]. The RFLP procedure was performed as described by Bidwell et al. using P32-labelled recombinant probes for DRB, DQB, and DQA [2].

PCR procedure

A one-step PCR-SSO [6, 17], mainly based on the procedure proposed at the XIth International Histocompatibility Workshop (IHWS), was used [5, 7]. In brief, DNA was extracted from peripheral blood lymphocytes or spleen cells using a salting-out method [8]. The PCR reaction consisted of 33 PCR cycles in a Biomed 60 thermal cycler with the following primer pair: GH46 [18] without restriction site and DRB-AMP-B primer as proposed at the XIth IHWS [5]. A classical dot blot was performed and hybridized with 15 oligonucleotides (1001, 1003, 1004, 1005, 1006, 1007, 1008, 1009, 2808, 3703, 5703, 5704, 7004, 7007, 7001) chosen from the XIth IHWS [5], as well as with two locally prepared SSO covering DR 8 (-0805) (5'GTGTCCACCAGGGCCCGC3' . .L. . at AA74) and DR 1303 (Hag) (5'GGCCCGCTTGTCTTCCAG3' . .DK. . at AA 70,71). These 17 SSOs covered all equivalents of the serological specificities. Subtyping for DR2 was not performed. DR2 was typed on the DRB5 gene only. The oligonucleotides were labelled with digoxigenine ddUTP at the 3' end using terminal transferase (Boehringer, Mannheim, Germany). All membranes were hybridized simultaneously at 42 °C using 6 × SSPE, 5 × Denhardt, 0.1 % Na Sarcosine, 0.02 % SDS . Stringency washes were performed in 3 M tetramethyl ammonium chloride at 57 °C for all membranes. The results were visualized using antidigoxigenine-conjugated alkaline phosphatase and color precipitation with NBT/X phosphate.

Results

Comparison of serology with RFLP results

A total of 345 recipient and 273 donor samples were sent to the CTS Laboratory and 588 RFLP results were received on 326 kidney graft recipients and 262 donors. For 19 recipients and 11 donors, no RFLP results were made available; these were not analyzed further. In total, 1176 antigens were tested by both serology and RFLP.

Serology results that were identical with the RFLP results were considered correct and therefore, not retested by a second DNA typing method. For 88 antigens in 84 individuals (14.3 %), the RFLP and serology results were discrepant. In 4 individuals (3 recipients and 1 donor) 2 mistyped HLA-DR antigens were found (Table 1).

Table 1 Individuals with two incorrect DR antigens by serology. The numbers between brackets in the first column indicate the CTS registry identification number

Serology	RFLP	PCRSSO
DR1/DR15 (17838)	DR10/DR14a	DR10/DR14
DR3/DR4 (15801)	DR103 or DR1/DR11.1[a]	DR103/DR11
DR7/DR13 (15348)	DR11.1/DR2LUM	DR11/DR16 new
DR1/DR5 (6402)	DR8/DR13b2[b]	DR8/DR1102
DR4/DR– (10588)	DR7 or DR9/DR8[c]	DR9/DR8

[a] DR1 (DRB1*0101 or DRB1*0102) and DRBR (DRB1*0103) cannot be distinguished by RFLP
[b] After PCR-SSO and PCR-SSP, this sample was not retained as a double error
[c] In some cases, DR 7 and DR 9 cannot be distinguisted by RFLP

Transcription errors

A number of serology/RFLP discrepant results were due to transcription errors. As these errors originated from the transmission of retrospective data from the laboratory to the CTS registry or vice versa, they had no impact on the matching degree between patient and kidney donor. They accounted for 2.2 % of the discrepancies (7 in 326 recipients and 6 in 262 donors). The errors were reported to the CTS registry where the results were corrected (Table 2).

RFLP/Serology discrepancies

The RFLP results were considered as reference results for the purpose of this analysis. The evaluation of discrepancies, therefore, was relative to the RFLP allele assignments. If only one RFLP pattern was found, the cell was considered homozygous for the proposed HLA-DR specificity and counted twice when calculating allele frequencies (e.g., a DR 3/DR blank by serology is given as DR17.1/DR17.1 by RFLP if only one RFLP pattern is found).

There were 84 wrongly typed individuals: 64 of 326 recipients and 20 of 262 donors. Before confirmation analysis by PCR-based DNA typing, this corresponded to a discrepancy rate of 19.6 % in the recipients and a lower 7.6 % in the donors (Table 2).

DR1. DR1 was assigned by RFLP 146 times. Sixteen times (10.9 %), however, serology gave a different re-

Table 2 HLA-DR Discrepancies between RFLP and serology

Before retyping with a PCR-based method	Recipients	Donors	Recipients + donors
Discrepancies between HLA-DR serology and RFLP	64/326 (19.6 %)	20/262 (7.6 %)	4/588 (14.3 %)
transcription errors	7/326 (2.1 %)	6/262 (2.2 %)	13/588 (2.2 %)
All discrepancies found; transcription errors included	21.7 %	9.8 %	16.5 %
After retyping with a PCR-based method			
Discrepancies confirmed by PCR-SSO and/or PCR-SSP, INNO-LiPA	62/326 (19.0 %)	18/262 (6.8 %)	80/588 (13.6 %)
Discrepancies *not* confirmed by PCR-SSO and/or PCR-SSP, INNO-LiPA	2/326 (0.6 %)	2/262 (0.8 %)	4/588 (0.7 %)
Discrepancies confirmed; transcription errors included	21.1 %	9.0 %	15.8 %

Table 3 Retyping results of the discrepancies found between RFLP and PCR-SSO in this series of 588 individuals. PCR-SSP and INNO-LiPA DRB are used as a third confirmatory DNA method. The numbers between brackets indicate the CTS registry identification number. The results in italic are confirmed incorrect DNA typing results

HLA DR Serology	RFLP DRB	INNO-LiPA	Generic PCR-SSO	PCR-SSP DRB1	Consensus typing
DR5/DR13	*DR11.1/DR11.1* (10570)	DRB1*1102/1305 or DRB1*11/1301	DRB1*11/13 DRB3*0301 or DRB3*02	11/13.1	under evaluation
DR11/DR	*DR11.1/DR13b2* (6420)	DRB1*11/1102	DRB1*11/1102	11/–	DR11/DR1102
DR11/DR	*DR8/DR13b2* (6402)	DRB1*08/1102	DRB1*08/1102	08/11	DR08/DR1102
DR3/DR11	*DR13b1/DR17.2* (15827)	DRB1*03/1102	DRB1*1102/03	03/11	DR03/DR1102
DR7/DR13	DR11/DR2LUM (15348)	*DRB1*11/1103* DR2 Group specific Amplification DR 16	*DRB1*11/–* DR2 Group specific Amplification: DRB1*16	11/16	DR11/DR16 DR16 without DRB5 gene

10570, 6420, 6402, and 15827: PCR-SSO confirmed by PCR-SSP.RFLP incorrect
15348: RFLP confirmed by second step PCR-SSO

sult. After PCR-SSO confirmation, the serological errors proved mostly ($n = 6$) to be due to overestimation of a second allele in homozygous DR1 individuals. HLA-DR 103 ($n = 10$ in this population) was not recognized and gave a DR-blank result.

DR2. DR2 ($n = 169$) was correctly typed in most cases. Only 4 errors were found in 169 cases (2.3 %), one of which involved a rare variant haplotype.

DR3. DR3 ($n = 147$) was missed ten times (6.3 %). Seven of the ten cases involved DR3/DR3 homozygous individuals (based on RFLP patterns). Seven times a second antigen was overestimated by serology and not confirmed by RFLP; it was mostly a DR7. This was probably due to crossreactions of DR7 sera with DQ2 sera.

DR5. DR5 was identified 156 times ($n = 141$ as the DR11 and $n = 15$ as the DR12 subtype). Fifteen of the 156 DR5 antigens (9.6 %) were incorrectly typed.

DR6. Our results regarding DR6 ($n = 192$) are concordant with previous data [23] showing the highest percentage of errors in this antigen group. We found that 24 of 106 DR6-positive recipients and 6 of 86 DR6-positive donors were incorrectly typed (30/192, or 15.6 %). In ten cases, the RFLP alleles DR13b1 (equivalent to DR 1303 or Hag) or DR13b2 (probably equivalent to DR1102) were involved (Table 3). In 9 cases a DR14 was missed by serology, and in 11 cases a DR13 was missed. DR14 was not recognized as DR6 by serology in 9 of 37 cases (24.3 %).

DR7. DR7 appeared 158 times in this population. No DR7 was missed by serology. However, DR7 was overestimated seven times as an extra reaction, mostly in homozygous DR3 individuals.

DR8. DR8 appeared 34 times. In five cases it was not recognized by serology.

DR9. DR9 appeared seven times. It was missed two times and typed correctly by serology five times.

DR10. DR10 appeared only 13 times among the 1176 antigens tested. Twelve of those were correctly identified by serology.

To summarize, the highest error rates were found for DR1 (10.9 %), DR5 (9.6 %), and DR6 (15.6 %).

Investigation of discrepant results by PCR-SSO

The 84 individuals who revealed a discrepant HLA-DR typing after comparing serology and RFLP were retested with a one-step PCR-SSO technique.

In 79 of these 84 individuals (94.4 %), PCR-SSO typing confirmed the RFLP result. In 5 individuals, however, PCR-SSO typing revealed a discrepant result when compared to the RFLP method, and these individuals were, therefore, re-evaluated with a third DNA typing method: PCR-SSP and/or INNO-LiPA reverse dot blot.

Confirmed discrepancies between RFLP and serology

The discrepancies found between serology and RFLP could be confirmed by PCR-SSO typing for 82 of the 88 discrepant antigens.

Forty-six incorrect serological antigen assignments were found in RFLP-confirmed heterozygous individuals; 22 of the 82 serological errors were found in RFLP homozygous individuals due to the overestimation of a second antigen by serology. This was most frequently seen for RFLP DR3 or DR1 homozygous typing results. For DR3 it involved mostly an overestimation of DR7. A second antigen recognized by RFLP in serologically "homozygous" individuals accounted for 14 of the 82 serological typing errors. The second antigens found by RFLP were: DR13 or DR14 ($n = 6$), DR103 ($n = 4$), DR1 ($n = 1$), and DR9 ($n = 2$).

Analysis of discrepant RFLP/PCR-SSO results (Table 3)

In five individuals PCR-SSO typing revealed a discrepant result when compared to the RFLP method. The typings were, therefore, re-evaluated with a third DNA typing method: PCR-SSP and/or INNO-LiPA reverse dot blot.

DR5/DR6 group. In four samples (6420, 6402,10570, 15827), the RFLP typing was incorrect and the serological typing was confirmed by PCR-SSO.

DR13b2, as determined by RFLP, proved to be a DRB1*1102 by the PCR-SSP and PCR-SSO methods. This specificity is equivalent to DR11 by serology. The allele appeared four times in this series as recognized by PCR-SSO (sample nos. 6420, 6402, 10570, and 15827). The PCR-SSP results confirmed the PCR-SSO results, which resulted in the conversion of all DR13b2 cases in the CTS file to DR11.4. DR13b1 and 13b2 differ in one RFLP band, either at 11.1kb (13b1) or at 13.0kb (13b2), which we believe to encode the DRB3*0101 and DRB3*02 alleles, respectively.

For sample 10570, a DR13 was found by PCR-SSP and PCR-SSO as well as by serology. RFLP, however, gave a DR11.1/DR11.1 homozygous pattern. INNO-LiPA indicated either the presence of DRB1*1305/1102 or DRB1*1301/1101(04). Additional testing on DRB1*1305 individuals in the CTS laboratory revealed that DRB1*1305 is indistinguishable from DR11 at the RFLP level. Sample 10570, however, remains under evaluation as PCR-SSP could not confirm the DRB1*1305/1102 combination.

Indistinguishable heterozygous RFLP patterns. Even after DQ typing, indistinguishable heterozygous RFLP patterns exist for DR17.1/DR11.1 and DR17.2/DR13b1. This resulted in some serological DR11 being assigned incorrectly by RFLP as DR13, or vice versa (e.g., sample 6427). DR7.1/DR4.1 and DR9.2/DR4.1 also give indistinguishable RFLP patterns. One individual who was serologically assigned DR4/DR9 (sample 10173) was confirmed by PCR-SSO, whereas the RFLP result indicated a DR4/DR7.

DR2 variant. Sample 15348 was found to carry a rare DR2 haplotype, typed by RFLP as a DR15. But unlike a classical DR15, it was assigned in RFLP as DR15.2, similar to DR 2LUM. However, this DR2 allele was missed by serology and one-step PCR-SSO. PCR-SSO missed the DR2 haplotype because our PCR-SSO system was based on hybridization with a DRB5-specific SSO (1009 of the XIth IHWS). As this particular DR2 haplotype was lacking a DRB5 gene, it could not be detected by our one-step PCR-SSO typing. Inclusion of SSO 1002 (DRB1-DR2) and a group-specific DRB1-DR2 amplification confirmed the presence of a DRB1-DR2 gene and hybridized to DNA oligoprobes specific for DRB1*16. The PCR-based techniques thus confirmed the presence of a DR2 allele but could not confirm the DR 2LUM (DRB1*1501 DRB5*absent), and RFLP remains discrepant in the final subtyping of this allele. Unfortunately, the two additional family members available for testing (one son and one brother) did not share this antigen with the patient.

Implications of donor/recipient matching

Considering the confirmed and corrected RFLP data, we found an overall discrepancy rate of 13.6 % between serology and DNA typing. Nineteen percent of the errors were found in recipients and 6.8 % in donors (Table 2). The 2.2 % transcription errors are not included in these percentages.

The 82 incorrect serological antigen assignments in 80 individuals resulted in 45 recipients with a change in the degree of donor/recipient HLA matching. In only six cases was a better match achieved, based on the corrected typing result. All others ($n = 39$) had a worse HLA match based on the DNA result than based on serological typing. Nine of the changes were due to an erroneous typing in the donor, whereas 30 were due to erroneous recipient typing (Fig. 1).

Discussion

Evaluation of donor and recipient typing results

Although much effort has been made to improve serological HLA class II typing, error rates in the order of 25 % have been reported [7]. In the CTS study, HLA-

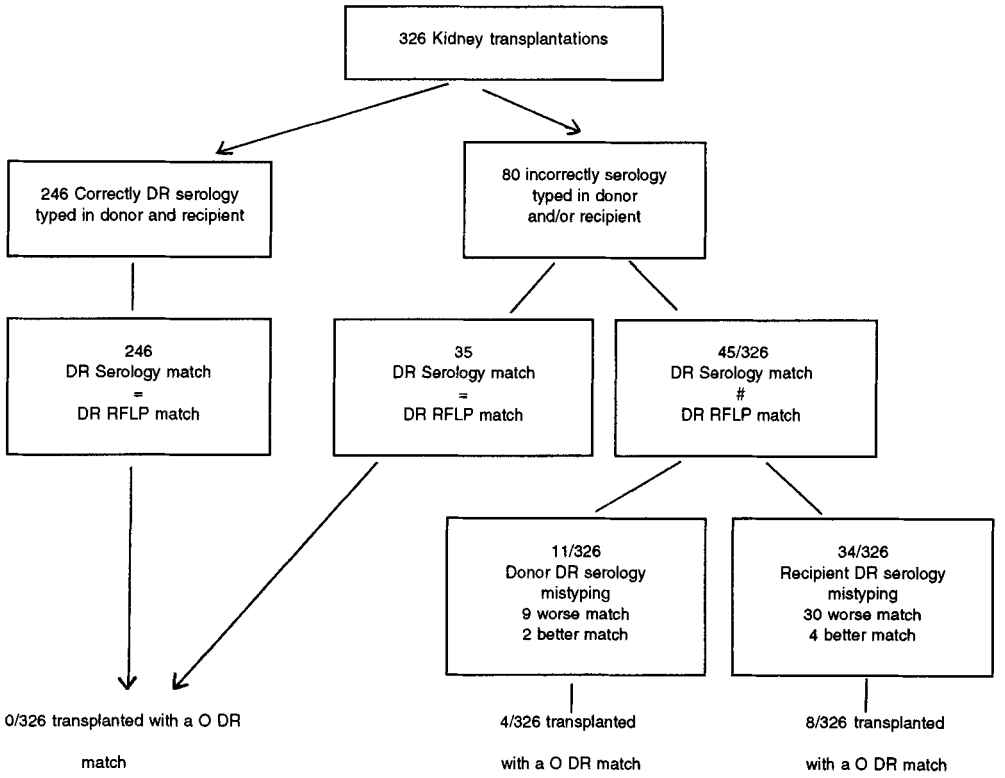

Fig. 1 Implications of donor/recipient matching

DR serology was compared to RFLP typing and revealed a discrepancy rate of 25 % for organ donors and 27.9 % for recipients. In our retrospective local study analyzing the CTS results of kidney transplantations performed between January 1988 and May 1992, those results were partly confirmed. We also found a relatively high discrepancy rate of 15.8 % for DR typing (2.2 % transcription errors included). However, separate analysis of the donors and recipients showed that these discrepancies were greatest among the recipients (21.1 %). The discrepancy rate for donor typing was a relatively low 9.0 %. The better serological typing result for donor typing was probably due to the better cell quality of spleen cells used for the typing of donors. Serological HLA-DR typing within the Eurotransplant area is nearly exclusively performed on donor spleen cells, which are known to have a high class II antigen expression. The difference in the discrepancy rates of donors and recipients is not surprising, as it is known that hemodialyzed patients are in an immunosuppressed state with a low expression of class II molecules on their cell membrane, which makes serological typing very difficult. Moreover, many recipients who received transplants between 1988 and 1991 (300 of 345) were HLA typed before 1988, when class II serology was still of poor quality. For "broad" HLA-DR antigens in retyped donors, Verduyn et al. [23] described a concordancy rate of 97 % between PCR-SSO and serological retyping of donor spleens, both performed in the Eurotransplant reference laboratory. In that study, however, only donor retypings performed in 1991 were evaluated. Our own retrospective study revealed a donor typing concordancy rate of 91.0 % between RFLP and serology (93.5 % if transcription errors are excluded).

Discrepancies between RFLP and PCR-SSO

For the majority of samples, the RFLP data could be confirmed by the PCR-SSO technique. Some discrepancies between RFLP and PCR-SSO were due to indistinguishable RFLP patterns, even after DQ typing. Some HLA-DR subtypes could be correctly assigned only by PCR-SSO. However, RFLP was superior to PCR-SSO typing in the identification of homozygous individuals.

DR1 versus DR103

Unlike RFLP, a one-step PCR-SSO was able to differentiate between DRB1*0103 and DRB1*0101(02) in most cases (except DR1/DR13 or DR1/DR4 heterozygous combinations). Evaluating these PCR-SSO data, we found that the serologically missing DR1 proved to be a DRBR (DRB1*0103) in 10 of the 11 cases.

*DRB1*1303*

DRB1*1303 (DR 13 Hag by serology and DR 13b1 by RFLP) was correctly assigned by RFLP. It was also recognized by PCR-SSO. It appeared ten times in this series and was only twice recognized correctly by serology as a DR13, DQ7. The eight remaining cases were serologically typed as DR12, DR11, or DR3.

After clarification of the doubtful RFLP DNA typings by PCR-SSO and confirmation with PCR-SSP or INNO-LiPA, the correct DNA typing results were transmitted to the CTS registry. Indistinguishable Taq 1 RFLP patterns such as DR11.1/17.1 = 13b1/17.2, DR1 = DR103, DR7.1 = DR 9.2 are intrinsic problems of identical RFLP patterns of the Taq1 polymorphism. They were not retained as real typing errors in this analysis.

Real discrepancies

Only five real discrepancies were documented between RFLP and PCR-SSO. In four cases it was an erroneous RFLP result involving either DRB1*1102 assigned as DR13 or a DRB1*13 variant under evaluation assigned as DR11. In one case it was an erroneous PCR-SSO result, the missed DR2 variant.

We proved in this study that RFLP results are accurate and reliable for the assignment of DR antigens. Transcription errors still remain a problem. However, this type of error is, in all likelihood, identical for all registries; in our study, it accounted for approximately 2.2 % of errors only (Table 2). The newer PCR-based typing techniques (PCR-SSO, PCR-SSP, and reverse dot blot) identify the allele more directly and may be suitable methods for prospective donor genotyping. They can also give supplemental information on split specificities. RFLP may still be used for recipient typing and retrospective donor typing, as it remains a very good tool for the retrospective evaluation of HLA typing and especially for the confirmation of homozygosity.

Impact on HLA matching

We found a change in the degree of final donor/recipient match in 45 of the 80 discrepant cases. In the literature [10] it is reported that transplantations with two DR mismatches have a higher incidence of rejection. Also, DR matching has been shown to be important in the early post-transplant period and to be correlated with transplant-related morbidity [21]. Considering the matching policy of Eurotransplant, kidney transplants with two DR mismatches should not be performed. Twelve of 326 transplantations ended up with zero DR matches due to mistyping of either the donor ($n = 4$) or the recipient ($n = 8$). The surprisingly low frequency of typing discrepancies in the donor population resulted in a small number of transplantations in which the donor mistyping was responsible for a change to a worse HLA match grade (9 of 326). A prospective donor DNA typing technique could have prevented four of these nine recipients from receiving transplants with a complete HLA-DR mismatch, assuming that the DNA typing results were always correct. This argues against a hasty conversion to prospective donor DNA typing.

Moreover, although not yet evident from this study, the recent developments in molecular biology have had an important effect on serological typing results due to the possibility of cross comparisons. As long as HLA matching is based on the criteria of broad antigen matching with a negative crossmatch, we believe that more experience needs to be gained in routine DNA typing before these typing techniques, with their own pitfalls, are introduced for routine prospective donor typing. For recipients, however, routine DNA typing improves the quality of HLA-DR typing and, thus, the reliability of the HLA match at the time of transplantation. Therefore, DNA typing should be implemented for recipients as a routine test prior to transplantation.

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