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DNA typing: an important step forward?

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Abstract. In a collaborative project which was supported by 96 transplant centers, DNA typing of HLA-DR antigens was carried out on over 7,000 transplant donors and recipients at 8 participating laboratories. Approximately 25% of the individuals were found to have been typed incorrectly by serological means. An analysis of over 2,500 first cadaver kidney transplants showed a significant correlation of matching for the HLA-DR antigens in transplants where the serological typing was confirmed by DNA typing. In transplants where the serological typing was found to be incorrect, the analysis of serological HLA-DR mismatches resulted in no correlation with graft outcome whereas a significant correlation was found when the corrected DNA typed HLA-DR antigens were analyzed. Transplants which had been reported to the Collaborative Transplant Study based on serological typing as matched for HLA-A, -B, -DR or HLA-B, -DR were found to have a superior graft survival rate only if HLA-DR compatibility was confirmed by DNA typing.

Key words: DNA typing – Kidney transplantation – HLA matching

The application of DNA techniques allows a more accurate determination of HLA-DR alleles than the conventional serological technique [1, 2, 5, 7, 9]. We employed DNA typing to evaluate in a collaborative project the utility of this new technique for clinical histocompatibility matching. With the cooperation of 96 transplant centers, DNA typing of over 7,000 donors and recipients of kidney transplants was performed using the RFLP technique described by Bidwell et al. [1].

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Materials and methods

Peripheral blood lymphocytes or spleen tissue from transplant recipients or donors was obtained at the transplant centers and frozen at −20°C. The cell material was shipped on dry ice to the study center at the University of Heidelberg where DNA was extracted by routine methods [4, 6]. Aliquots of DNA were distributed to the participating laboratories where DNA typing was performed. The laboratories were not aware which DNA samples belonged to recipients or donors. The typing results were reported to the study center for further analysis. The serological HLA typings obtained in the individual transplant centers' laboratories were used for comparison. Information on the transplants was collected within the framework of the Collaborative Transplant Study. Graft survival rates were computed by the Kaplan-Meier method. Statistical significance was estimated by log rank or weighted regression analysis [3]. The transplants were performed between 1988 and 1990. Only first transplants from cadaver donors were included in the analysis.

Results

Table 1 shows the rates of discrepancies between serology and DNA typing for the HLA antigens HLA-DR1 to HLA-DRw10. Serological typing errors were particularly frequent for "difficult" antigens, i.e. specificities for which monospecific serological reagents are difficult or im-

Table 1. Discrepancies RFLP versus serological HLA-DR typing (7265 individuals typed)

Allele	*n*	% Discrepancies
DR1	1532	14.5
DR2	2227	7.6
DR3	1829	8.4
DR4	2342	8.5
DR5	1839	14.2
DRw6	2237	32.0
DR7	1393	7.7
DRw8	510	27.8
DR9	15	33.3
DRw10	142	40.1

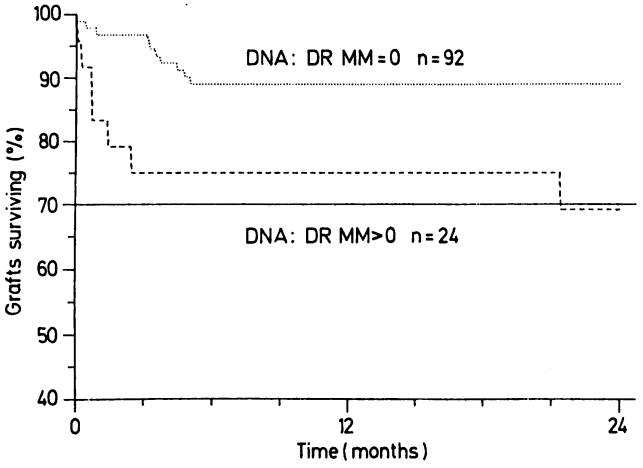

Fig. 1. Graft survival analysis of first cadaver kidney transplants that were reported, based on serological typing, to be HLA-A, -B, -DR compatible. Graft survival was significantly better if HLA-DR compatibility was confirmed by DNA typing as compared to grafts where DNA typing revealed an HLA-DR mismatch ($P < 0.02$)

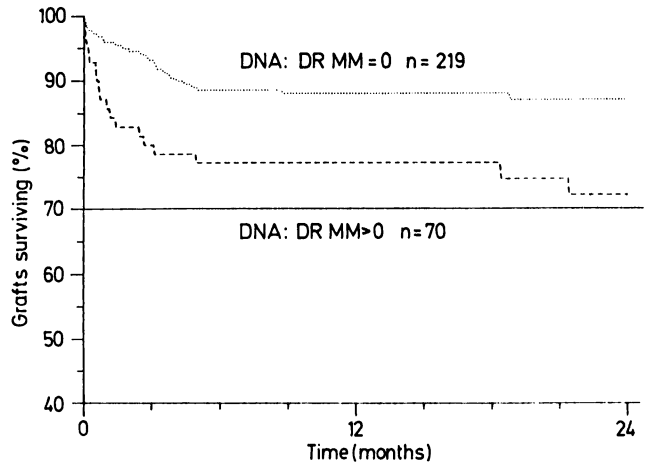

Fig. 2. Analysis of first cadaver transplants that were reported, based on serological typing, to be HLA-B, -DR compatible. Grafts in which HLA-DR compatibility was confirmed by DNA typing did significantly better than grafts with HLA-DR mismatches revealed by the DNA technique ($P < 0.01$)

possible to obtain. The error rate was approximately equivalent in recipient and donor typings.

The influence on the graft survival analysis of incorrect HLA-DR assignments based on serological typing is shown in Table 2. Whereas a significant effect of matching for HLA-DR was observed in transplants where both the recipient and donor typing were confirmed by the DNA technique, there was no correlation of matching with graft outcome when DNA typing revealed that the serological typing was incorrect. When the incorrectly typed transplants were analyzed according to the HLA-DR typings obtained by the DNA method, a significant correlation of HLA-DR matching with graft outcome became apparent.

We investigated the influence of incorrect HLA-DR assignment based on serological typing on the graft survival analysis of transplants that were reported to the Collaborative Transplant Study as matched for HLA-A, -B, and -DR. As shown in Fig. 1, transplants in which HLA-DR compatibility was confirmed by DNA typing had a significantly higher success rate than transplants in which DNA typing revealed HLA-DR mismatches ($P < 0.02$). In other words, even though all transplants had been considered "matched" based on serological HLA typing, only those in which the HLA-DR types were confirmed by the DNA technique had a superior graft outcome.

The analysis was extended to the "next best" match category, transplants matched for HLA-B, -DR (and mismatched for HLA-A). Even in this comparison, a significantly better graft survival rate was seen when the HLA-DR match was confirmed by DNA typing as compared to when DNA typing revealed an HLA-DR mismatch ($P < 0.01$) (Fig. 2).

Table 2. Effect of HLA-DR mismatches on graft survival; first cadaver transplants. Graft survival at 1 year (% ± SE)

Number of HLA-DR mismatches	Serology confirmed by DNA	Serology different from DNA	
		Analysis of serological mismatches	Analysis of DNA mismatches
0	84 ± 1 ($n = 765$)	82 ± 2 ($n = 321$)	87 ± 3 ($n = 120$)
1	82 ± 1 ($n = 995$)	80 ± 2 ($n = 304$)	81 ± 2 ($n = 358$)
2	78 ± 3 ($n = 255$)	81 ± 4 ($n = 79$)	78 ± 3 ($n = 226$)
P regression	0.02	ns	0.05

Discussion

DNA typing provides a powerful tool for the exact determination of HLA-DR allels. Our results demonstrated that when the serological errors were corrected, the correlation of HLA matching with graft outcome was improved. The implications for clinical histocompatibility matching are obvious.

In the current project we used frozen cell material in a retrospective study to evaluate whether accurate DNA typing would be of benefit in the clinical setting. With the answer at hand, it will now be important to introduce DNA techniques for prospective typing and allocation of organs. The RFLP method used in this project is unsuitable for that purpose because it requires approximately 1 week to obtain results. For DNA typing to become competitive, the time requirement will have to be shortened to approximately that of serological typing (about 5 h). New DNA techniques, based on the use of the polymerase chain reaction (PCR) and hybridization with oligonucleotides appear to rapidly close the time gap. Typing with sequence-specific oligonucleotides or the PCR-RFLP technique reduces the time requirement to approximately 10 h [9, 10]. The newest and most exciting development in this regard is the allele-specific amplification method employing sequence-specific primers described by Olerup

and Zetterquist [8]. This elegant technique allows the identification of HLA-DR alleles in approximately 3 h. It remains to be seen how soon this technique can be transferred from the research laboratory to the routine transplant laboratory setting. With a quick and accurate DNA technique at hand, the prospect for improved clinical histocompatibility matching appears good. If the rapid development in the DNA field during the last couple of years can be used as a guideline, improved matching and thus better transplant success rates should become reality in the foreseeable future.

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