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Discrepancies in serological tissue typing revealed by DNA techniques

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Abstract. DNA techniques were applied to typing a population of renal patients in order to assess the number of discrepancies between this technique and the serological technique. Five patients had been given an incorrect type by serology, and in 22 instances allogenotypes were found where previously there had been a serological blank. DNA typing was also able to determine allogenotypic subtypes that correlated with DR antigens difficult to split by serology and to determine allogenotypic subtypes correlating with DR antigens not known to have a split by serology. Whereas DNA typing provided a result first time of testing on all but 3 patients, the average number of serological testings for each patient was 1.9.

Key words: DNA typing - Discrepancies - Serological typing - Allogenotypes - Renal.

HLA typing is traditionally performed according to immunological methods using antibodies reacting with HLA gene products on the cell surface. These methods rely on obtaining monospecific alloantisera from multiparous females. In addition, for class II typing, suitable B lymphocyte preparations are required.

It is now possible using a single enzyme (TaqI)/single blot/multiple reprobe system for HLA-DR and -DQ [3] to assign the HLA-DR and -DQ specificity at the DNA level. We have applied this technique to typing renal patients in order to assess any differences obtained by this technique when compared to conventional serological methods.

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Materials and methods

The renal population consisted of 160 patients either transplanted consecutively since May 1986 or awaiting a transplant. The serological typing was by a 2-stage microlymphocytotoxicity test using B lymphocytes separated by sheep cell rosetting. The following antigens could be detected: HLA-DR1, -DR2, -DR3, -DR4, -DR5, -DRw6, -DR7, -DRw8, -DR9, and -DRw10, but splits of HLA-DR5 and DRw6 could not be detected. No typing was performed for HLA-DQ antigens.

The DNA methods have been previously described [7] with the exception that the electrophoresis was for 18 h at 40 V. Membranes were hybridised sequentially with the following radiolabelled cDNA probes: HLA-DRβ pRTV1 [2], HLA-DQβ pII-β-I [6] and HLA-DQα pDCH1 [1].

Results

Figure 1 shows the allogenotypes revealed using the DRβ cDNA probe pRTV1. The allogenotypes DRβ1, β4, β7, β8, β9, β10, β11, β12, β13, β14, β15, β16, β17, and βBr are associated with the corresponding HLA-DR serological specificities [3]. TaqI/DRβ allele-specific signals (allogenotypes) of 11.1 kb and 13.0 kb correlate respectively with the supertypic specificities DRw52a and DRw52b, two subtypes of DRw52 previously described [4].

Different allogenotypes were observed that correlate with an individual DR specificity. We found two allogenotypes, $\beta7^1$ and $\beta7^2$, in patients

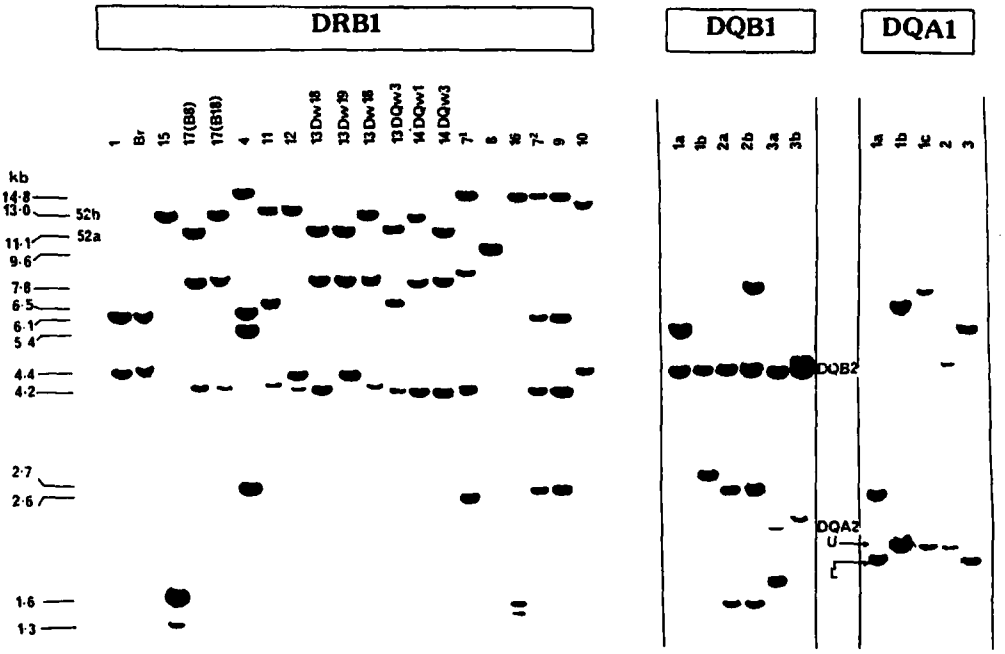

Fig. 1. RFLP patterns of representative cells with the DRβ1, DQβ1 and DQα1 probes

serologically typed as HLA-DR7. In patients previously typed as DRw6, we detected allogenotypes corresponding to DRw13-DQw1-Dw18 and DRw13-DQw1-Dw19. In addition, patients who are DRw13-DQw1-DQw18 can be differentiated by allogenotyping based on a polymorphism associated with DRw52 subtypes (Fig. 1). Similarly, two allogenotypes with a polymorphism associated with DRw52 were detected in patients serologically typed as HLA-DRw17 (Fig. 1).

It is not possible using this method to differentiate DRβ1 from DRβBr, DRβ7[2] from DRβ9, DRβ13-DQw1-Dw18 (DRw52a associated) from DRβ14-DQw3, and DRβ17 (DRw52b associated) from DRβ13-DQw1-Dw18 (DRw52b associated). Assignment of HLA-DRBr was given if the sera identifying HLA-DR1 were negative. Likewise, assignment of HLA-DRw9 was given if the sera identifying HLA-DR7 were negative, and assignment of DRβ13-DQw1-Dw18 (DRw52b associated) was given if the sera identifying HLA-DRw17 were negative. Assignment of DRβ13-DQw1-Dw18 (DRw52a associated) was given if, after reprobing with the DQ probes, the DQ allogenotype was either 1a or 1b.

Figure 1 also shows representative results of rehybridising with the DQβ and DQα cDNA probes, respectively. The allogenotypes identified and their correlation with DQ serotypes have previously been described [3].

When the results obtained by allogenotyping were compared to those obtained by serology, there was a difference in 27 (17%) of the patients. In 5 patients, an incorrect antigen had been assigned by serological typing. On two occasions DRw6 had been assigned instead of DR3, and on a further two occasions instead of DRw12. On one occasion

Table 1. Antigens not detected by serological typing

Antigen	Number of patients	
	Antigen not detected	Having antigen
DRBr	11	11
DRw8	2	5
DRw14-DQw1	2	7
DR9	1	1
DRw16	1	1
DRw10	1	2
DRw13-DQw1-Dw19	1	11
DR1	1	23
DRw17 (52a)	1	45
DR4	1	67
Total	22	

Table 2. Allogenotypes found associated with the same serological HLA-DR specificity

Antigen	Allogenotype	Patients with split	
		Number (n=160)	%
DRw17	DRβ17 (52a)	45	28.1
	DRβ17 (52b)	5	3.1
DR7	DRβ7[1]	18	11.1
	DRβ7[2]	15	9.4
DRw13 (DQw1)	DRβ13 (DW18-52a)	3	1.9
	DRβ13 (DW18-52b)	5	3.1
	DRβ13 (DW19)	11	6.9

DRw12 was assigned instead of DRw13-DQw3. In 22 patients, an antigen detected by allogenotyping was not detected by serology (Table 1). The patient positive for DRw16 was also positive for DRw15 (Table 1).

The 160 patients required serological typing on an average of 1.9 occasions. Ninety patients were typed on one occasion, 34 patients on two occasions, 17 patients on three occasions, 9 patients on four occasions, 5 patients on five occasions and 5 patients on six occasions. Using DNA typing, we found that 27/160 (17%) of our renal patients were homozygous.

The frequency of the different allogenotypes found associated with the same serological HLA-DR specificity are shown in Table 2. In these allogenotypic subtypes, HLA-B8 was found in the phenotype of 41/45 patients with DRβ17 (52a), but never in the phenotype of 5 patients with DRβ17 (52b). In contrast, HLA-B18 was found in 3/5 patients with DRβ17 (52b), but never in patients with DRβ17 (52a).

In the 15 patients who were positive for $DR\beta7^2$, HLA-B13 was present in 4 patients, HLA-Bw50 in 3 patients and HLA-Bw57 in 6 patients, but these antigens were absent in patients with $DR\beta7^1$. In contrast, HLA-B44 was present in 11/18 patients with $DR\beta7^1$ and only 2/15 patients with $DR\beta7^2$ and HLA-B14 in 5/18 patients with $DR\beta7^1$ and only 1/15 patients with $DR\beta7^2$.

Discussion

The methods described in this report permit the assignment of HLA-DR and -DQ allogenotypes by the sequential use of short DRβ, DQβ and DQαDNA probes. The DRβ, DQβ and DQα allogenotypes in heterozygotes are interpreted by the summation of patterns demonstrated by homozygous control cells.

A major application of DNA typing is in the determination of HLA-DR and -DQ allogenotypes when serological assignment is not possible or where the results are equivocal. Serological assignment is influenced by poor quality or low numbers of circulating B cells and by the lack of reliable monospecific alloantisera for certain specificities.

Our results have shown that the majority (22/27) of differences between the serological and allogenotyping methods are due to the detection of allogenotypes on occasions when there was a serological blank. The remaining five discrepancies arose due to the difficulties involved in the serological typing of DRw13, an antigen for which it is difficult to obtain monospecific sera.

The problems that we incurred in serological typing were due to the non-availability of suitable sera. This is a reflection on the difficulties in obtaining sera for the uncommon specificities and not on our sera screening or exchange programme. Indeed, we actively exchange sera with more than 100 laboratories. On all occasions, when differences between the allogenotype and the serological type are found, it is possible using the allogenotype result to assess the quality of the HLA sera. Thus, by using DNA techniques, we were able to define the correct tissue type of all our renal recipients. Further advantages are that we were able to identify allogenotypes associated with splits of HLA-DR5 and HLA-DRw6 and to define different allogenotypes correlating with the same serological specificity. Some of these allogenotypes are due to a different split of DRw52 in association with the DR type. We can now assign DQ-associated allogenotypes using DNA techniques, whereas we could not assign DQ specificities by serological means because all the DQ sera available to this laboratory are contaminated with DR antibodies.

A further advantage of using DNA typing is that we only had to obtain a repeat sample for DNA typing on three occasions in contrast to the average number of times each patient was typed serologically. The difficulties with the serological typing were mainly due to poor preparations of B lymphocytes. There were also occasions when a type was repeated because an antigen was suspected but could not be completely proven to be present. It was only on retyping serologically that we were able to prove definitely that the antigen was present.

Using DNA typing meant that we were able to prove homozygosity without resorting to the extra typings of family members. The importance of the correct type and the strength of association between matching and graft survival have been previously shown [5, 8]. Indeed, when a matching effect has not been found, one explanation given has been the high number of patients with only one antigen detected [9].

DNA techniques have already proved useful in this laboratory. As the technique is developed, it should be possible to identify further splits of class II antigens and their relevance in transplantation.

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