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HLA class II DNA-RFLP typing in 102 individuals from Northern Greece

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Abstract The serological identification of HLA class II alleles is often doubtful. Since accurate HLA typing is essential for the matching of donor-recipient pairs in allogeneic transplantation, an effort was made to establish DNA restriction fragment length polymorphism (RFLP) typing and to assess the correlation between the serological and RFLP techniques in the population of Northern Greece. One hundred and two healthy individuals (204 HLA-DR alleles) from Northern Greece were HLA-DR, DQ typed with both the microcytotoxicity and the Taq I RFLP method, using three exon-specific probes. DNA-RFLP typing revealed (1) concordant results with serology in 69.9% (142/204) of the alleles and (2) at least one HLA-DR allele discrepant to serology in 30.4% (62/204) of the alleles. Incorrect serological DR types (weak reactions or inability to distinguish between two alleles with a common epitope) were identified in 54 alleles (26.5%), while 3.9% (8/204) of serological "blank" alleles turned out to be definable alleles by RFPL. Of the individuals tested, 10.8% (11/102) were DR-homozygous by RFLP. This comparison of results obtained by serology and RFLP demonstrated the necessity of the clinical application of DNA typing, especially for organ transplantation where accurate HLA typing has an important influence on graft survival.

Key words HLA antigens
DNA-based typing techniques
Restriction fragment length polymorphism (RFLP)

Introduction

The HLA system is a highly polymorphic region, located on the short arm of chromosome 6, containing genes coding for at least three different classes of proteins: HLA class I, class II and class III antigens [1]. The analysis of their polymorphism is necessary to facilitate successful organ transplantation [2]. In addition, these loci have been implicated in susceptibility to autoimmune and other diseases and, furthermore, they contribute greatly to anthropological studies and paternity testing [3].

Identification of HLA class II antigens is conventionally performed by serological (reactivity with allo- or monoclonal antisera) and cellular (mixed lymphocyte culture or primed lymphocyte test) techniques. Nevertheless, it is known that these conventional techniques often cause problems due to weak reactions, inability to distinguish between two alleles with a common epitope, or purity and viability of B lymphocytes DNA restriction fragment length polymorphism (DNA-RFLP) analysis of HLA class II antigens has been shown to be a more accurate and reliable method [4]. The involvement of our

boratory in tissue typing of donor-recipient pairs in logeneic transplantation led us to establish the DNA-FLP analysis in order to obtain more accurate results, correlate the results obtained by the serological and RFLP methods and to assess the true frequency of HLA class II antigens in the population of Northern Greece (Epirus, Macedonia and Thrace). Our participation in the international Collaborative Transplant Study (CTS) and the CTS experience in the field of molecular typing encouraged us in our efforts to establish the DNA-RFLP technique.

Materials and methods

Blood samples (heparinized blood for serological typing and EDTA blood for preparation of DNA) were obtained from 102 unrelated, healthy individuals from Northern Greece. All individuals were HLA-DR typed by the standard microlymphocytotoxicity assay, using at least 3 mono- or oligospecific antisera per HLA-DR specificity.

The DNA-RFLP method was performed according to the method presented at the Fourth European Histocompatibility Conference by J. Bidwell. In brief, isolated DNA from nucleated cells was digested with the restriction endonuclease Taq I, followed by agarose gel electrophoresis, denaturation and Southern transfer of single-stranded DNA from the gel to a nylon membrane. Membranes were then hybridized sequentially with the following radiolabelled cDNA probes: HLA-DRβ pRTV1 [5], HLA-DQβ pII-β-1 [6] and HLA-DQα pDCH1 /7].

Results

Figure 1 shows the autoradiogram of 15 individuals whose genomic DNA was digested with Taq I and probed with the pRTV1 DRB probe. The DRβ allogenotypes defined by this probe correlated with serologically assigned DR specificities. In addition, various DRβ splits of the HLA-DR antigens were determined. Figure 2 shows

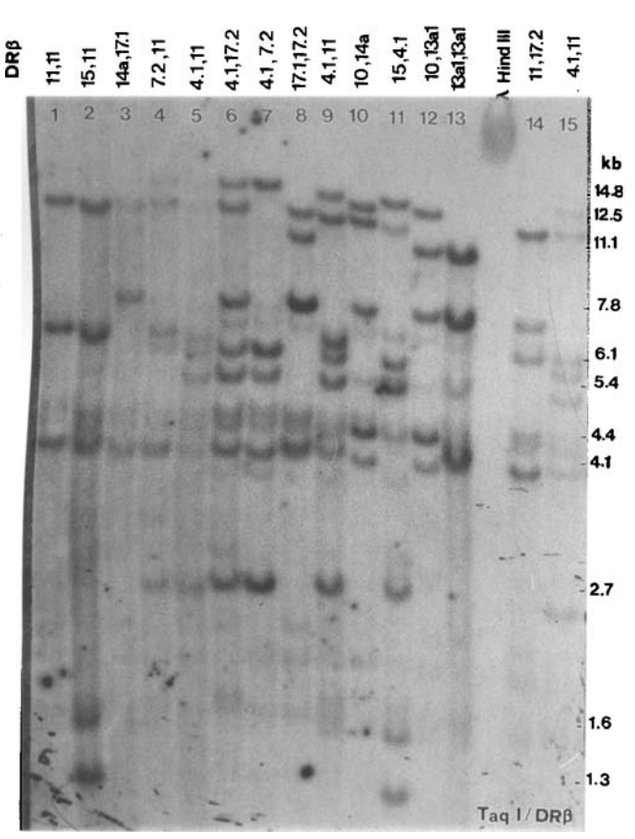

Fig. 1 Autoradiogram of 15 individuals whose genomic DNA was digested with Taq I and probed with the pR TV1 DRB probe. DRB-RFLP patterns and sample numbers are shown on the top of each track, while fragment sizes are given in kilobase pairs (*kb*) at the right side of the autoradiogram

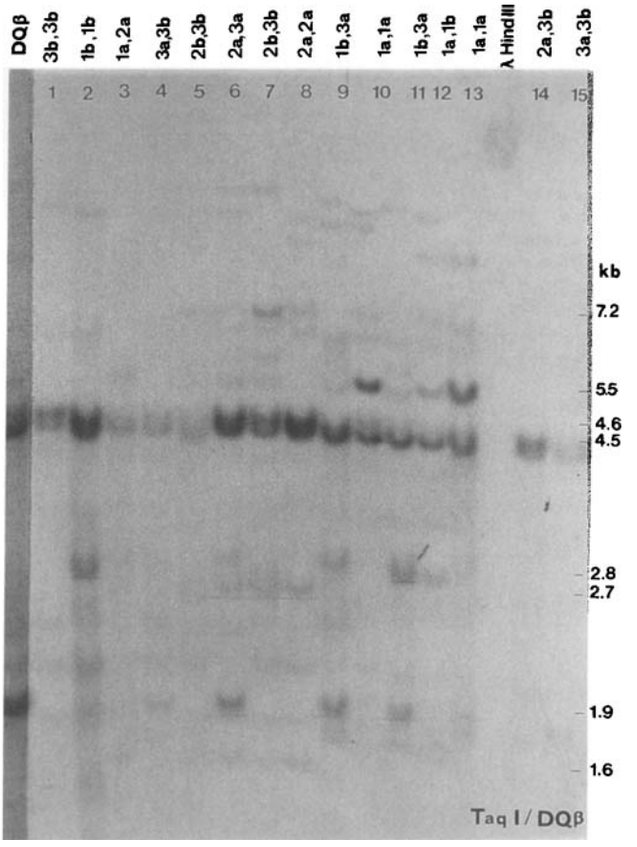

Fig. 2 The DNA samples were rehybridized with the pII-β-1 DQB probe. DQB-RFLP patterns and sample numbers are shown on the top of each track and fragment sizes (*kb*) at the right side of the autoradiogram

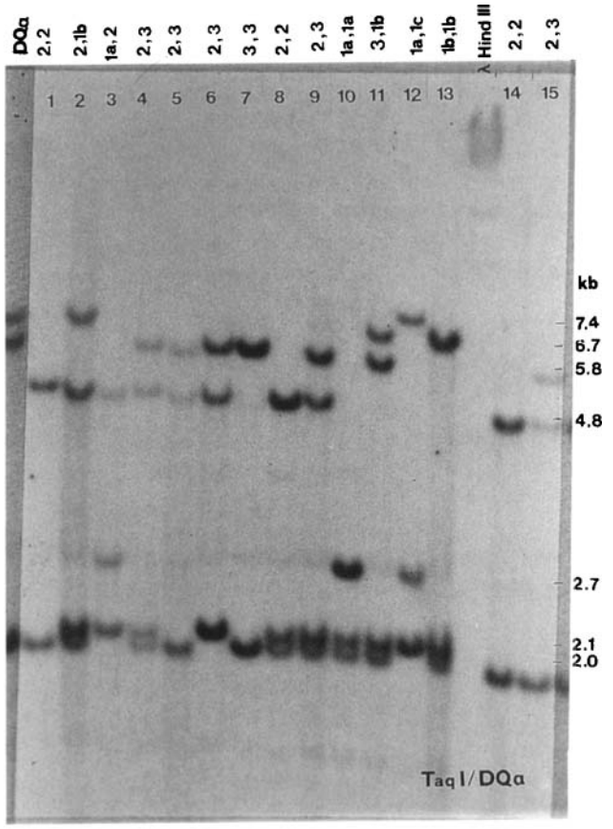

Fig. 3 The same DNA samples were reprobed with the pDCH1 DQA probe. DQA-RFLPs and sample numbers are shown on the top of each track and fragment sizes (*kb*) at the right side of the autoradiogram

the results of rehybridizing the membrane shown in Fig. 1 with the DQB cDNA probe. Using this probe, seven RFLPs were identified that correlated with splits of DQ serological types. Figure 3 shows five DQα RFLPs of the same DNA samples probed with the DQα probe.

The HLA-DRβ, DQβ and DQα gene and antigen frequencies of 204 alleles are listed in Table 1. The terminology of Bidwell [8] is used followed by the associated serological specificities for easy comparison. Only previously described Taq I DRβ, DQβ and DQα fragment patterns were seen. No unusual Taq I DRβ-DQβ-DQα associations were observed. In addition, normal frequencies for the various DRβ splits of the antigens were determined.

To compare the RFLP results with frequencies of DR alleles in Northern Greece, we translated the Taq I DR alleles to the corresponding serological alleles (Table 2). The discrepancies involved primarily certain antigens such as DR6, DR8 and DR10, antigens known to be difficult to type by serology. Besides, after RFLP typing, no blank alleles remained.

Comparison of the results obtained by serology and RFLP revealed: (1) concordant results with serology in 69.6% (142/204) of the alleles and (2) at least one HLA-DR allele discrepant from serology in 30.4% (62/204) of the alleles. Precisely, incorrect serological DR types were

Table 1 Gene and antigen frequencies of HLA-DRβ, DQβ and DQα alleles in a Northern Greek population

RFLP	HLA-DR serology	$n=204$ alleles	(%) Gene frequency	(%) Antigen frequency
HLA-DRβ				
DRβ1	DR1	14	6.86	13.72
DRβ15	DR15(2)	11	5.39	10.78
DRβ16	DR16(2)	14	6.86	13.72
DRβ17.1	DR17(3)	13	6.37	12.74
DRβ17.2	DR17(3)	8	3.93	7.86
DRβ18	DR18(3)	0	0.00	0.00
DRβ4.1	DR4	24	11.77	23.54
DRβ4.2	DR4	0	0.00	0.00
DRβ11.1	DR11(5)	46	22.55	45.10
DRβ11.2	DR11(5)	0	0.00	0.00
DRβ11.3	DR11(5)	0	0.00	0.00
DRβ12.1	DR12(5)	2	0.98	1.96
DRβ12.2	DR12(5)	0	0.00	0.00
DRβ13a.1	DR13(6)	9	4.41	8.82
DRβ13a.3	DR13(6)	4	1.96	3.92
DRβ13a.4	DR13(6)	1	0.49	0.98
DRβ13b1/2	DR13(6)	4	1.96	3.92
DRβ14a	DR14(6)	14	6.86	13.72
DRβ14b	DR14(6)	4	1.96	3.92
DRβ7.1	DR7	0	0.00	0.00
DRβ7.2	DR7	11	5.39	10.78
DRβ7.2/9	DR7/9	8	3.93	7.86
DRβ8	DR8	6	2.94	5.88
DRβ10	DR10	11	5.39	10.78
Total		204	100.00	200.00
HLA-DQβ				
DQβ1a	DQ5, DQ6(1)	57	27.94	55.88
DQβ1b	DQ6(1)	20	9.80	19.60
DQβx	DQ6(1)	2	0.98	1.96
DQβ2a	DQ2	21	10.30	20.60
DQβ2b	DQ2	12	5.88	11.76
DQβ3a	DQ4, DQ8, 9(3)	32	15.69	31.38
DQβ3b	DQ7(3)	60	29.41	58.82
Total		204	100.00	200.00
HLA-DQα	HLA-DQ			
DQα1a	DQ5(1)	39	19.12	38.24
DQα1b	DQ4, DQ5, 6(1), DQ7(3)	32	15.69	31.38
DQα1c	DQ6(1)	13	6.37	12.74
DQα2	DQ2, DQ7(3)	77	37.74	75.48
DQα3	DQ2, DQ7, 8, 9(3)	43	21.08	42.16
Total		204	100.00	200.00

Table 2 Comparison of HLA-RFLP and serological gene frequencies (%) in the Northern Greek population

HLA-DR	RFLP $n=102$	DR-serology $n=102$	DR-serology $n=449$[a]
DR1	6.86	6.93	9.0
DR2	12.25	12.37	16.0
DR3	10.30	9.90	10.6
DR4	11.77	13.86	12.1
DR5	23.53	23.76	28.9
DR6	17.64	9.90	10.0
DR7	9.41	11.38	8.6
DR8	2.94	0.99	0.3
DR9	0.99	0.99	0.5
DR10	5.39	0.00	1.2
DRx	–	9.90	9.6

[a] Serological frequencies were obtained in a previous study

Table 3 Discrepancies between RFLP-DRβ typing and serological HLA-DR typing ($n=204$)

No discrepancy	69.6%	(142/204)
Antigen/antigen	26.5%	(52/204)
Antigen/"blank"	3.9%	(8/204)
RFLP homozygous	10.8%	

identified in 26.5% (54/204) of the alleles, while 3.9% (8/204) of serological "blank" alleles turned out to be definable alleles by RFLP. Of the individuals tested, 10.8% (11/102) were DR-homozygous by the RFLP method (Table 3).

Discussion

Considering the need of an international waiting list for organ or bone marrow transplantation as well as a list of bone marrow donor volunteers, it is necessary to improve and standardize existing HLA typing methods. The demonstration that approximately 25% of serological HLA-DR typings can be erroneous compared to DNA analysis calls for the introduction of DNA typing techniques [4]. Moreover, a significant difference in graft outcome has recently been shown in a retrospective study between DNA HLA-DR matched and mismatched transplants [2].

The RFLP technique was the first method used to identify HLA class II polymorphisms at the DNA level. Although it has major disadvantages (slowness, radioactivity), our results revealed an important qualitative difference between DNA typing and serology in the population of Northern Greece. The analysis of incorrect serological DR types (30.4%) showed that most discrepancies involved crossreactivities between alleles with a common epitope (26.5%), while 3.9% of alleles were defined that had been incorrectly assigned as "blanks" by serology. In addition, we noticed that the majority of the discrepancies occurred when serotyping was performed between 1986 and 1989. Better quality HLA-DR antisera and more efficient techniques for B lymphocyte preparations (B Lymphokwik, immunomagnetic beads) introduced more recently may explain the improvement in our results after 1989.

RFLP typing is a useful technique for obtaining HLA frequencies in a normal population. Consequently, it might provide an insight into genetic relationships among different ethnic groups. RFLP can reveal unknown alleles, resulting in new linkage disequilibria between DNA fragments and the formation of "natural selection haplotypes" [9].

The frequencies of Taq I/DRβ RFLPs, when compared with those obtained with serological typing in a previous study (Table 2), revealed a very impressive discrepancy rate for certain alleles. The discrepancies concerned mainly DR6, DR8 and DR10 antigens, for which monospecific antisera are still difficult to obtain. For DR6, the gene frequency appears to be increased from 10.00% by serology to 17.64% by RFLP (Table 2). Consequently, this frequenc incorrect assignment might explain the presence or absence of a "DR6 effect" in kidney transplantation [10, 11].

The HLA-DRβ frequencies were also compared with reported frequencies in other European populations based on the data of the 11th International Histocompatibility Workshop. No particular difference between the Greek population and other European ethnic groups was revealed. This is important information in view of plans for international organ and bone marrow exchange lists.

Despite the limitations demonstrated by RFLP typing, which were related to the slowness of the technique, we intend to continue the use of Taq I/DR allogenotyping for the typing of bone marrow and kidney transplantations. Meanwhile, technical improvements in DNA-based typing are already available. Therefore, we have adopted DNA typing using the PCR method whereby the time required for HLA-DR typing has been dramatically shortened, allowing us to use DNA typing also for cadaver donors.

References

1. Hansen T, Sachs D (1989) The major histocompatibility complex. In: Paul W (ed) Fundamental Immunology, 2nd edn., pp 445–487
2. Opelz G, Mytilineos J, Scherer S et al for the Collaborative Transplant Study (1991) Excellent survival of HLA matched cadaver kidney transplants defined by DNA HLA-DR typing. Lancet 338:461–463
3. Polymenidis Z (ed) (1990) Immunological aspects '90. pp 324–337
4. Mytilineos J, Scherer S, Opelz G (1990) Comparison of RFLP-DR beta and serological HLA-DR typing in 1500 individuals. Transplantation 50:870–873
5. Bidwell JL, Jarrold EA (1986) HLA-DR allogenotyping using exon specific cDNA probes and application of rapid minigel methods. Mol Immunol 23:1111–1116
6. Larhammar D, Schenning L, Rask L et al (1982) Complete amino acid sequence of an HLA-DR antigen like β chain as predicted by the nucleotide sequence: similarities with immunoglobulins. Proc Natl Acac Sci USA 79:3687–3691
7. Auffray C, Lillie JW, Arnot D et al (1984) Isotypic and allotypic variation of class II histocompatibility antigen α chains. Nature 308:327–333
8. Bidwell JL, Bignon JD (1990) European Histocompatibility Conference, Strasbourg
9. Polymenidis Z, Adam K, Parapanissiou E et al (1992) Importance of certain HLA-A, B haplotypes for the survival of renal grafts from living-related donors. Transplant Proc 24:2458–2460
10. Mytilineos J, Scherer S, Dunckley H et al (1993) DNA typing of 3500 cadaver kidney transplants does not confirm the "DR6 effect". Transplant Proc 25:207–209
11. Adam K, Polymenidis Z, Papakyriazi L (1992) HLA-A, B, DR antigens and renal transplantation from cadaveric donors and living-related donors in Greek patients. Transplant Proc 24:2449–2450