Letter to the editor

Transplant International

Transpl Int (1993) 6: 58–59

HLA-DR typing of organ donors and allograft recipients by SSO typing: correlation with serotyping in the North Italy Transplant Program

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Received March 20, 1992/Accepted June 22, 1992

Sir: In many histocompatibility laboratories, including ours, HLA-DR typing by standard serology is not satisfactory [1, 6, 7]. Sometimes difficulties are encountered in obtaining suitable B-lymphocyte-enriched suspensions from transplant candidates and organ donors, and the interpretation of results is often equivocal.

To compare the results obtained by serology with those by sequence-specific oligonucleotide typing (SSO typing), we performed the latter on 393 subjects from whom blood samples had been taken and stored and who had serological HLA-DR typing data. Of these, 201 were organ donors; the others had received a kidney ($n = 167$), a heart ($n = 21$), or a liver ($n = 4$) transplant between 1988 and 1991. All assays were performed at the tissue typing laboratory of the Reference Center of the North Italy Transplant Program.

Serological HLA class II typing was determined by microlymphocytotoxicity employing Collaborative Transplant Study or commercially available HLA-DR typing trays. B cells were isolated from peripheral blood or lymph nodes using immunomagnetic microspheres (Class II Dynabeads; Dynal, Oslo, Norway) [2, 8]. The following specificities were recognized: DR1, 2, 3, 4, 5, w6, 7, w8, 9, and w10.

SSO typing was performed following a protocol described by Giphart and Verduijn [3] on PCR-amplified DNA extracted by the standard chloroform/ethanol procedure or by the salting-out method [5]. The SSO probes used allowed typing of the following specificities: DR1, 2, 3, 4, 7, w8, 9, w10, w11, w12, w13, and w14. The reactivity pattern of these probes is described elsewhere [3].

It should be noted that all cases of SSO typing for DR5 and DRw6 subtypes were considered as a concordance when DR5 and DRw6 were assigned by serology.

Although no selection for doubtful cases was carried out and typings were performed in the same laboratory, comparison between serological and SSO typing results revealed a worrying rate of discrepancies. As shown in Tables 1 and 2, SSO typing was discrepant with serology in 27.7% of all subjects (30.2% of the recipients and 25.3% of the donors). When the incorrectly attributed alleles were considered, the percentage of discrepancies was 16.4% for recipients and 13.8% for donors. In eight cases both antigens assigned by serology were discrepant with SSO typing. In our experience, among the more frequent antigens in the population studied, DR5, DRw6, and DR7 were the specificities most frequently wrongly assigned by serology. A higher incidence of mistakes and of non-attributed antigens was observed in transplant recipients than in donors, for whom cells are obtained from the spleen or lymph node.

Table 1. Comparison between SSO typing and serology in 192 recipients. In five cases (not shown) both antigens assigned by serology were incorrect

SSO	Serology												Total[c]
	1	2	3	4	5	w6	7	w8	9	w10	x[a]	x[b]	
1	37		1			1	1				1	2	43
2	2	35									1	3	41
3			32			1					1	3	37
4				28	1		1					1	31
5					79								
(w11)	1	1		1	(73)	1	1				11	2	91
(w12)					(6)							1	7
w6						45							
(w13)			1		5	(32)	1				1	3	43
(w14)	1	1			2	(13)		2		1		1	21
7							33						33
w8					3	1		12				2	18
9									2				2
w10	1					1				3		2	7
Total[d]	42	37	34	29	90	50	37	14	2	4	15	20	374

[a] Apparently homozygous by serology, confirmed by SSO typing
[b] Apparently homozygous by serology, second antigen defined by SSO typing
[c] Number of times alleles were defined by SSO typing
[d] Number of times antigens were defined by serology

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Table 2. Comparison between SSO typing and serology in 201-donors. In three cases (not shown) both antigens assigned by serology were incorrect

SSO	Serology												Total[c]
	1	2	3	4	5	w6	7	w8	9	w10	x[a]	x[b]	
1	34			2	1	3	1					3	44
2		35			1	3					4	1	44
3			29			1					2		32
4		1	1	33			1				2	1	39
5					74								
(w11)			1	1	(69)	1					5	1	78
(w12)			1	1	(5)		2						9
w6						60							
(w13)				2	5	(47)					1	3	58
(w14)	1					(13)	1		1			1	17
7					1	1	53				4	1	60
w8					1			10					11
9							1						1
w10	1									2			3
Total[d]	36	36	32	39	83	69	59	10	1	2	18	11	396

[a] Apparently homozygous by serology, confirmed by SSO typing
[b] Apparently homozygous by serology, second antigen defined by SSO typing
[c] Number of times alleles were defined by SSO typing
[d] Number of times antigens were defined by serology

Finally, in our series, 33 subjects (8.4 %) were homozygous by both serology and SSO typing, a lower proportion than that found by authors who used restriction fragment length polymorphism [4, 6].

In conclusion, in our hands, SSO typing proved to be an accurate tool for HLA-DR typing, capable of overcoming the difficulties encountered with serology.

Acknowledgement. This study was supported by a grant from "Ricerca Corrente", Ospedale Maggiore, 1989, Progetto N. 51501.

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