Identification of HLA Alleles with Low or No Cell Surface Expression in the Czech Population

(HLA, Czech population / HLA-A*24020102L / DRB4*01030102N)

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Abstract. The presence of the A*24020102L allele is implicated in one donor from the CBMD who serologically was typed as A2; B44, B55; Cw1, Cw7. The DRB4*01030102N allele was identified in one healthy donor and in one patient with MDS during routine HLA class II DNA typing.

The DRB4*01030102N allele was identified in the patient's father, who had CML, and was associated with the HLA-A3-B7-Cw7-DRB1*0701-DQB1*0303 haplotype, which is common for European populations.

In order to avoid mistyping, both techniques, serology and molecular biology must be used for HLA typing, especially for cases where just one antigen appeared to be present using serological methods.

HLA class I antigens are still typed by serology by some bone marrow registries and histocompatibility laboratories involved in solid organ transplantation. Problems may arise when HLA antigens are typed by serological or DNA methods only. Serology can miss some antigens, while intermediate resolution DNA typing can incorrectly assign an antigen which is not actually expressed by a null allele. Although the frequency of HLA class I expression variants is not high, and was estimated as between 0.04% and 0.53% in different populations, such errors in tissue typing may have disastrous implications for allogenic transplants (Williams et al., 1997). A combination of serological and DNA-based genotyping methods including PCR-SSP, PCR-SSOP and sequence-based typing (SBT) facilitates the identification of HLA class I null and low-expression alleles, although allele-specific typing is necessary for their final definition (Bunce et al., 1995).

The detection of HLA class II antigens is mainly provided by DNA-based typing, but identification of null alleles happens in routine practice rather by chance, when allele-specific typing is performed. In particular, this involves *DRB3*, *DRB4* and *DRB5* genes encoding the DR52, DR53 and DR51. Under the current HLA nomenclature null alleles for *DRB4* and *DRB5* exist and their number is steadily increasing (Marsh et al., 2002). So far, it is known that three genes of the *DRB4* locus do not express the DR53 protein on the cell surface (DRB4*01030102N, DRB4*0201N and DRB4*0301N). Nucleotide sequence analysis of DRB4*01030102N revealed a single base substitution in the acceptor splice site at the 3' end of intron 1, changing the AG dinucleotide to AA. The nucleotide sequences of all the exons and the remaining splice junctions are identical to those of the "normal" *DRB4* gene (Sutton et al., 1989). The DRB4*01030102N allele seems to be exclusively present on the DRB1*07-DQB1*0303 haplotype (O'Neill et al., 1996), although an unusual association with DRB1*04-DQB1*0302 has been reported recently (Gassner et al., 1999; Voorter et al., 2000).

Material and Methods

Serological and DNA typing for HLA class I antigens has been carried out in 264 donors from the Czech Bone Marrow Donor Registry (CBMD), 77 patients from IKEM solid organ transplantation waiting list and 200 healthy individuals. Samples from individuals were selected for DNA typing where only one HLA-A antigen and two HLA-B antigents and/or two HLA-A antigens and one HLA-B antigen were detected by serology.

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Abbreviations: CBMD – Czech Bone Marrow Donor Registry, CML – chronic myelogenous leukaemia, MDS – myelodysplastic syndrome, SBT – sequence-based typing.

Table 1. HLA-A sequencing primers

Primer	Exon	Direction	Location	Sequence (5'→3')
5AIn1-99	exon 2	forward	intron 1:99-119[1]	AgCCgCgCCKggASgAgggTC
3AIn2-37	exon 2	reverse	intron 2: 37-57	ggCCCgTCCgTgggggATgAg
5AIn2-150	exon 3	forward	intron 2: 150-171	gTTTCATTTTgRgTTKAggCCA
3AIn3-41	exon 3	reverse	intron3: 41-66	AAACSgCCTCTgYggggAgAAgCAA
5A24	exon 4	forward	intron 3: 483-502	TTCTgTgCTCYCTTCCCCAT

[1]intron numbering is based on individual introns

Serological typing of HLA class I antigens was performed by the standard complement-dependent lymphocytotoxicity test (Terasaki and McClelland, 1964) or by using the Histo Tray ABC 120 trays (Biologische-Analysensystem-GmbH, Lich, Germany). DNA extraction was performed by salting out (Miller et al., 1988). Automated reverse PCR-SSOP (low resolution LiPA, Innogenetics, Gent, Belgium) and PCR-SSP technology (low and high resolution Olerup SSP GenoVision VertriebsmbH kits, Vienna, Austria) were used for HLA class I and class II DNA typing. In this report, the first detection of A*24020102L and DRB4*01030102N alleles in the Czech population is described.

Results and Discussion

The presence of the A*24 null or low expession allele was implicated in one donor from the CBMD. His phenotype was A2; B44, B55; Cw1, Cw7 (determined by serology), DRB1*1301, DRB1*16011, DQB1*0603, DQB1*0502. Allele-specific PCR-SSP (Olerup SSP HLA-A9 kit) demonstrated the presence of an A*24020102L allele. Trays with five different local polyclonal antisera and commercial trays with two polyclonal antisera (A23+A24) and one monoclonal antiserum (A24) failed to detect this antigen. SBT was used to confirm the results. The complete HLA-A gene was amplified using the locus-specific primers for the entire gene, which were published by Domena et al. (1993). Details of amplification conditions have been described (Bunce et al., 2000). Sequencing of exons 2, 3 and 4 was performed using primers shown in Table 1, with BigDye™ cycle sequencing kits (Applied Biosystems) using an ABI PRISM 3100 Genetic Analyzer. Sequence data was analysed and HLA allele assignment was performed using Sequencing Analysis™ and MatchTools™ software programmes (Applied Biosystems).

A*24020102L has an identical sequence to that of A*24020101 except for a single nucleotide substitution (A instead of G) at position 244 in intron 2 (position 708 according to HLA-A Genomic Sequence Alignments (http://www.ebi.ac.uk/imgt/hla/align.html). The mutation at the 3' end of intron 2 affects the correct splicing of this intron so that only very low levels of mature protein are expressed (typically < 5%) and are almost serologically invisible (Laforet et al., 1997; Zanone-Ramseier et al., 1999). They are sufficient, however, to stimulate an alloreactive T-cell response (Magor et al., 1997). The sample had two nucleotides, guanine and adenine (R = G + A) at this position 708 (G from A*020101 and A from A*24020102L), which confirmed the presence of A*24020102L allele.

Following the recent publications showing that the B*55-Cw*01 haplotype seems to be associated with the A*24020102L allele (Dunn et al., 2003), we selected four samples among 541 individuals studied with this haplotype. A*24020102L was present in just one case.

DRB4*01030102N is another allele with an unusual level of protein expression that was identified in the Czech population. The DRB4*01030102N allele was identified in one healthy donor and in one patient with MDS during routine HLA class II DNA typing. Family studies showed the presence of the DRB4*01030102N allele in the patient's father, who had CML (Table 2). The DRB4*01030102N was associated with the A3-B7-Cw7-DRB1*0701-DQB1*0303 haplotype, which is common for European populations.

*Table 2. HLA haplotypes in the family of a patient with DRB4*01030102N*

	HLA-A	HLA-B	HLA-C	DRB1*	DQB1*	DRB
Patient (with MDS)	A3	B7	Cw7	0701	0303	4*01030102N
	A1	B27	Cw2	1502	0602	5*010101
Father (with CML)	A3	B7	Cw7	0701	0303	4*01030102N
	A3	B7	Cw7	1501	0602	5*010101
Mother	A29	B44	Cw2	0701	0202	4*01010101
	A1	B27	Cw2	1502	0602	5*010101
Sister	A3	B7	Cw7	1501	0602	5*010101
	A1	B27	Cw2	1502	0602	5*010101

Our results demonstrated that a new HLA antigen typing strategy should be established for the transplantation programme. In order to avoid mistyping, both techniques, serology and molecular biology must be used for HLA typing, especially for cases where just one antigen appeared to be present using serological methods.

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