Short Communication

HLA-A, HLA-B and HLA-C Polymorphism in the Slovak Population

(HLA class I typing / PCR-SSP / occurrence rates of HLA-A, -B and -C alleles)

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Abstract. The occurrence rates of class I HLA alleles were investigated in a sample of the Slovak population by a PCR-SSP method. The frequencies of HLA-A alleles ranged from 0.00 for A*4301 to 0.2798 for A*0201-22; the frequencies of HLA-B alleles ranged from 0.00 for B*4601, B*4801-3, B*5901, B*7301, and B*8101 to 0.1101 for B*4402-10, and those of HLA-C alleles from 0.00 for Cw*1301 and Cw*1402-3 to 0.2661 for Cw*0701-10. The occurrence rates of class I HLA alleles established in our study were compared with those in the Czech population. No significant differences were found.

HLA class I typing by standard serological techniques is associated with a lack of reagents for assignment of some antigens and an incomplete typing due to a low level of resolution within a cross-reactive group. The result of this situation is a high frequency of blanks. The introduction of DNA-typing methods to the study of HLA polymorphism has dramatically changed the situation. These techniques are much more precise and the resulting HLA polymorphism is much greater (Bodmer et al., 1999).

We report the results of HLA class I typing by polymerase chain reaction-sequence specific primers (PCR-SSP) in a sample of the Slovak population, as only serologically defined phenotype frequencies of HLA antigens have been available so far (Nyulassy et al., 1977; Královičová et al., 2000).

Material and Methods

Blood samples were obtained from 109 unrelated random blood or bone marrow donors who resided in Slovakia (Bratislava region). In accordance with their surnames, they were of Slovak origin.

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Abbreviation: PCR-SSP – polymerase chain reaction-sequence specific primers.

DNA was isolated from peripheral blood leucocytes. The polymorphism of HLA class I alleles was investigated by the PCR-SSP method using Dynal HLA-A, -B and -C "low resolution" kits (Dynal, Oslo, Norway).

DNA extraction

DNA was isolated from peripheral blood leucocytes obtained from EDTA anti-coagulated blood. DNA was prepared by a salting-out method (Miller et al., 1988).

Amplification conditions

Dynal HLA-A, -B and -C low-resolution-SSP primer sets contained 5'- and 3'-primers for identification of the A*0101 to A*8001, B*0702 to B*8201, and C* 0102 to C*1802 alleles. Twenty-four PCR reactions for HLA-A, 48 for HLA-B and 18 for HLA-C were performed per sample. Each tube in the set contained a primer solution consisting of a specific primer mix, i.e. an allele- and a group-specific primers as well as a control primer pair matching non-allelic sequences. PCR reaction mixtures consisted of 110 ng DNA, 5 µl PCR-solution 10× (PCR buffer-GIBCO (London, UK), 50 mM $MgCl_2$, 200 µM of each dNTP, glycerol, cresol red), 0.4 i.u. of Taq-polymerase (GIBCO, London, UK) and 5 µl of a primer mix. PCR amplifications were carried out in a termocycler "PTC-100™-Programmable Comprised Thermal Controller" (MJ Research, Inc., Watertown, MA). The cycling parameters were as follows: 2 min at 94°C, followed by 10 cycles of 10 s at 94°C, and 60 s at 65°C, 20 cycles of 10 s at 94°C, 50 s at 61°C and 30 s at 72°C.

Gel electrophoresis

Electrophoresis of PCR products was performed in 1% agarose gels containing 0.5 µg/ml ethidium bromide. Gels were run for 15 min at 15 V/cm in 0.5× TBE buffer and visualized under UV illumination.

Gene frequencies of HLA-A, -B and -C loci were determined by direct calculation, and significance of compared data was calculated by the χ^2-test.

Table 1. Occurrence rates of HLA-A, -B and -C alleles in the Slovak population (the highest frequencies are in bold characters)

HLA-A		Frequency		HLA-B		Frequency		HLA-C		Frequency	
n = 109	No.	f	g	n = 109	No.	f	g	n = 109	No.	f	g
A0101-2	32	0,2936	0,1468	B0702-8	22	0,2018	0,1009	Cw0101-3	13	0,1193	0,0596
A0201-22	61	0,5596	0,2798	B0801-4	19	0,1743	0,0872	Cw02021-3	12	0,1101	0,0550
A0301-3	30	0,2752	0,1376	B1301-4	10	0,0917	0,0459	Cw0302-9	19	0,1743	0,0872
A2301	7	0,0642	0,0321	B1401-2	4	0,0367	0,0183	Cw0401-6	34	0,3119	0,1560
A2402-14	20	0,1835	0,0917	B1501-37	11	0,1009	0,0505	Cw0501-2	9	0,0826	0,0413
A2501-2	8	0,0734	0,0367	B1801-5	17	0,1560	0,0780	Cw0602,4	16	0,1468	0,0734
A2601-8	12	0,1101	0,0550	B2701-11	13	0,1193	0,0596	**Cw0701-10**	58	0,5321	0,2661
A1101-4	13	0,1193	0,0596	B3501-21	18	0,1651	0,0826	Cw0801-4	7	0,0642	0,0321
A2901-3	3	0,0275	0,0138	B3701-2	5	0,0459	0,0229	Cw1202-6	32	0,2936	0,1468
A3001-4	6	0,0550	0,0275	B3801-2	10	0,0917	0,0459	Cw1301	0	0,0000	0,0000
A31012	3	0,0275	0,0138	B3901-12	3	0,0275	0,0138	Cw1402-3	0	0,0000	0,0000
A3201-2	7	0,0642	0,0321	B4001-10	7	0,0642	0,0321	Cw1502-6	3	0,0275	0,0138
A3301-3	1	0,0092	0,0046	B4101-2	3	0,0275	0,0138	Cw1601-4	4	0,0367	0,0183
A3401-2	2	0,0183	0,0092	B4201-2	1	0,0092	0,0046	Cw1701-2	5	0,0459	0,0229
A3601	2	0,0183	0,0092	**B4402-10**	24	0,2202	0,1101	Cw1801-2	2	0,0183	0,0092
A4301	0	0,0000	0,0000	B4501	1	0,0092	0,0046	Cx	4	0,0367	0,0183
A6601-3	2	0,0183	0,0092	B4601	0	0,0000	0,0000				
A6801-3	3	0,0275	0,0138	B4701-2	2	0,0183	0,0092				
A6901	2	0,0183	0,0092	B4801-3	0	0,0000	0,0000				
A7401-3	3	0,0275	0,0138	B4901	3	0,0275	0,0138				
A8001	1	0,0092	0,0046	B5001-2	4	0,0367	0,0183				
Ax	0	0,0000	0,0000	B5101-9	11	0,1009	0,0505				
				B5201	2	0,0183	0,0092				
				B5301-2	1	0,0092	0,0046				
				B5401	1	0,0092	0,0046				
				B5505	3	0,0275	0,0138				
				B5601-3	4	0,0367	0,0183				
				B5701-4	6	0,0550	0,0275				
				B5801-2	3	0,0275	0,0138				
				B5901	0	0,0000	0,0000				
				B6701	1	0,0092	0,0046				
				B7301	0	0,0000	0,0000				
				B7801-2	1	0,0092	0,0046				
				B8101	0	0,0000	0,0000				
				B8201	1	0,0092	0,0046				
				Bx	7	0,0642	0,0321				

f – allele frequency
g – gene frequency

Results

The obtained allele frequencies are shown in Table 1. The most frequent HLA-A alleles were A*0201-22 (0.2798), A*0301-3 (0.1376), and A*0101-2 (0.1468). The most common B alleles were B*4402-10 (0.1101), B*0702-8 (0.1009), and B*0801-4 (0.0872). Among HLA-C alleles the most common were Cw*0701-10 (0.2661) and Cw*0401-6 (0.1560). The least frequent alleles were A*4301 (0.00), B*4601, B*4801-3 (0.00), B*5901 (0.00), B*7301 (0.00), B*8101 (0.00), Cw*1301 (0.00), and Cw*1402-3 (0.00).

Discussion

The frequencies of HLA-A, -B and -C alleles established in the present study are in agreement with our previous results, in which HLA class I antigens were detected by a microlymphocytotoxic test (Ivašková et al., 1971; Nyulassy et al., 1977; Královičová et al., 2000). However, the more sensitive and exact PCR-SSP method allowed us to decrease the frequency of "blank" alleles (Table 2). No HLA-A blank alleles were detected, and those of HLA-B and HLA-C were 3.21% and 1.83%, respectively. The "blank" alleles include unidentified as