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Short Communication

Distribution of HLA-C Alleles Determined by PCR-SSP in the Population of Lower Silesia

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Abstract. We typed 100 unrelated, healthy Poles from the region of Lower Silesia for HLA-C using low-resolution polymerase chain reaction sequence-specific primers (PCR-SSP) and compared the observed allele frequencies with data published for other human populations. Poles appeared to be most similar to the Germans and English more distant from the French, Catalans and Basques and most dissimilar to non-Caucasians from Equatorial Guinea and Japan. It would be interesting to HLA-C-type other Slavic and non-Slavic peoples from Middle and Eastern Europe for comparison.

Key words: HLA-C; allele distribution; PCR-SSP typing; Lower Silesian Polish population.

Introduction

The human major histocompatibility complex class I antigen HLA-C is inadequately defined by serological methods: only 10 alleles have been detected, and up to 50% of individuals in a population may have only one detectable HLA-C allele or even both alleles undetected ("blank")[5]. This is in sharp contrast with HLA-A and -B alleles, which are accurately typed by serology, are extremely polymorphic, and their function in antigen presentation is well established[33]. One of the reasons for the unreliable determination of HLA-C by serology is its low cell-surface expression compared with that of HLA-A and -B[13, 16, 17, 24, 33].

Due to the poor definition of its alleles, the importance of HLA-C has, until recently, been neglected in the clinic, and even its biological role has been questioned[17]. However, improved detection and resolution of HLA-C alleles by molecular biological methods have shed light on the biological role of HLA-C. Indeed, HLA-C typing by molecular methods has already proved to be much more accurate than serological typing[4–6, 10, 21, 23]. There is growing interest in HLA-C because of its recently uncovered role in a number of immune phenomena. These include bone marrow graft failure[3, 26, 27], organ graft rejection[2], anti-viral response[8, 14, 19, 30], resistance to natural killer cells and to HLA-non-restricted cytotoxic T lymphocytes[11], and association with psoriasis vulgaris, which is one of the most frequent skin diseases in Caucasians[7]. Knowledge of HLA-C allele distribution in a given human population is therefore highly desirable, and has already been performed for several Caucasian and non-Caucasian populations.

Several techniques for the molecular typing of HLA-C have already been published, based on locus-

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-specific amplification in polymerase chain reaction (PCR) followed by the detection of restriction fragment length polymorphism[31], by hybridization with sequence-specific oligonucleotide probes[15, 18], or by sequencing[10, 27, 29]. In recent years commercial HLA-C low resolution PCR sequence-specific primers (PCR--SSP) have appeared on the market. We used such a system for HLA-C typing of our population, which had not been tested on the molecular level thus far.

Materials and Methods

Donors. One hundred unrelated healthy Polish people, mostly from the local Lower Silesian population, were tested. Ten ml of blood were collected into a tube containing EDTA and used fresh or frozen. DNA was extracted using the salting-out procedure described earlier[20, 22].

PCR-SSP. Sixteen HLA-C group-specific primer mixes (Dynal GmbH, Hamburg, Germany) were used. PCR was performed according to the manufacturer's recommendations. Reaction products were separated by gel electrophoresis (1.5% agarose, Sigma, in 1 × TBE buffer) for 10 min at 150 V (i.e. 15 V/cm). Subsequently, ethidium bromide (0.5 µl/µl) was added and the gel was examined under a UV transilluminator. The result was compared with the Interpretation Table supplied by Dynal. For HLA-Cw alleles, only the group designation (e.g. Cw*07) is given throughout the text, because differentiation among the alleles of a given group was not possible with this PCR-SSP system.

Results and Discussion

Genomic DNA from 100 healthy unrelated Polish donors was typed by PCR-SSP. Allele frequencies are shown in Table 1. For comparison, examples of published HLA-C allele frequencies in some other Caucasian as well as non-Caucasian populations, i.e. the English[4], German[23], and French[31], Catalan and Basque[9], Equatorial Guinean (namely, the Bubi people of island of Bioko)[25], and Japanese[1] data are also given in the table.

The HLA-C allele frequencies of Poles were close to those of the English[4] and Germans[23] (Table 1). Allele frequencies in the French population[31] were different from those of the Poles, Germans and English for several alletes. The frequencies of Cw*07 and Cw*16 in French people[31] were similar to the linguistically closely related Catalans[9]. However, the frequency of Cw*03 in French people, as published by TATARI et al.[31], was three times lower than that of other European populations, including the Catalan and Basque (Table 1). This finding requires a comment. TATARI et al.[31] typed 113 French individuals by HLA-C locus-specific amplification followed by multi-step restriction fragment length polymorphism (PCR-RFLP), whereas all other popula-

Table 1. HLA-C allele frequencies in healthy Polish subjects compared with other populations

HLA-C allele	Frequencies (%)							
	Poles (100)[a]	Germans (280)	English (604)	French (113)	Catalans (87)	Basques (99)	Bubi (100)	Japanese (167)
Cw*01	3.0	3.9	2.8	4.0	2.3	5.1	0.0	18.5
Cw*02	5.5	4.3	2.8	5.8	7.5	0.5	9.5	0.0
Cw*03	12.5	12.6	13.0	4.5	12.1	12.6	10.5	22.0
Cw*04	11.0	11.2	10.1	13.7	9.8	9.1	11.0	4.4
Cw*05	5.0	7.3	10.6	11.5	11.5	13.6	1.0	0.4
Cw*06	9.0	10.5	11.0	7.5	8.0	3.5	8.0	0.2
Cw*07	34.0	29.5	33.6	18.6	15.5	27.7	21.5	13.7
Cw*08	2.0	3.7	3.7	6.2	5.2	2.5	6.5	12.6
Cw*12	11.0	7.8	3.6	1.3	8.6	4.5	4.5	12.2
Cw*13	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Cw*14	0.5	1.2	1.1	3.5	5.2	0.5	4.5	11.6
Cw*15	1.0	2.8	2.2	4.9	3.4	3.0	0.5	3.6
Cw*16	2.0	3.4	4.2	10.2	10.3	16.6	10.5	0.0
Cw*17	3.5	1.2	0.8	0.0	0.6	0.0	9.5	0.0
Cw*18	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0

HLA-C allele frequencies (%) in 100 Poles as compared with other human populations from: Germany[23], England[4], France[31], Catalonia[9], the Basques region[9], the Bubi of Equatorial Guinea[25], and Japan[1].

[a] Number of individuals tested.

tions reported so far were typed by PCR-SSP or sequencing. Moreover, only Cw*0302, but not Cw*0303 or Cw*0304, was detected in the French population[31], whereas Cw*0302 is virtually absent from all other Caucasians[4, 9, 23], who possess rather Cw*0303 and Cw*0304. In addition, using the same assay system, the same French group typed a panel of lymphoblastoid cell lines and again found only Cw*0302, but not Cw*0303 or Cw*0304 allele[32], whereas other investigators found Cw*0304, but not Cw*0302, in those lines that were tested in their laboratories[12, 28]. There was no discordance in the other HLA-C alleles. Thus, we may suspect that something was wrong with the Cw*03 determination in French people and that if this population were retyped using more conventional method (i.e. PCR-SSP and/or sequencing), the HLA-Cw*03 frequency might appear more similar to that of other Europeans.

Frequency gradients for Cw*08, Cw*14 and Cw*15, with an increase from Poles through Germans and the English to the French and Catalans, and an opposite gradient for Cw*17, were observed (Table 1). The Basques, who are linguistically distant from Indo-Europeans, were different from other Caucasians, including Poles, in the frequencies of some HLA-C alleles[9]. Non-Caucasion populations from Africa[25] and Asia[1], shown here for comparison, were different from Caucasians in most allelic frequencies (Table 1).

It is worth noting that, with respect to HLA-DR*15 and DR*16 frequencies, Poles are more similar to Roumanians than to Western Europeans (Dr. Beata Nowakowska of our Institute, personal communication). It would then be interesting to compare the HLA-C phenotype and allele frequencies in our Polish population with those in other Slavic and non-Slavic Middle- and East-European populations. It is conceivable that the Polish population, which has been exposed throughout its history to both Western and Eastern admixtures, will be revealed to be genetically intermediate, as reflected in the distribution of polymorphic markers such as HLA alleles.

Although the Dynal PCR-SSP kit used here had a resolving power insufficient for donor-recipient HLA-C matching in unrelated bone marrow transplantation, it should prove useful in HLA-C typing for bone marrow transplantation from related donors and for organ transplantation, as well as in studies on the association of HLA-C with disease. Indeed, the accuracy of the determination of HLA-C alleles by PCR-SSP has recently been confirmed by sequence-based typing[10]. Its superiority over serological HLA-C typing has already been demonstrated by us[21] and others[10, 23].

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