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Determination of HLA-A*02 Alleles Using Nested PCR-SSP in Korean Population

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HLA-A2 is one of the most diversified HLA-class I antigen with 17 subtypes so far identified at the molecular level. HLA-A*02 subtyping has significant implications on the tissue typing for organ and bone marrow transplantations. Recently, DNA-based typing methods have been successfully applied to the elucidation of HLA gene polymorphisms. In the present study, HLA-A*02 genotyping was established by using nested polymerase chain reaction-sequence specific primers (PCR-SSP) and distribution of A*02 alleles were determined in Korean individuals. Genomic DNA prepared from four B-lymphoblastoid cell lines and lymphocytes from serologically defined 48 HLA-A2 Korean individuals by phenol/chloroform extractions was typed. The results of the four B-lymphoblastoid cells were consistant with the previous data typed by PCR analysis. Five A*02 alleles - A*0201, A*0203, A*0206, A*0207 and A*0210 - were commonly observed in a total of 17 A*02 alleles. Of these, A*0207 (f = 49.0%) was the most frequent allele in Korean population. A*0206 (f = 28.3%) and A*0201 (f = 17.0%) were also found frequently while A*0203 and A*0210 types were observed in less than 5%. In conclusion, the high level of discrimination for HLA-A*02 alleles will prove useful and informative in the study of transplant survival, and may identify the importance of allelic differences not readily detectable by serology on host and donor compatibility.

The HLA-A antigen is one of the Human Leukocyte Antigen (HLA) class I molecule encoded by three principal genes (A, B and C) within the HLA region of human chromosome 6. Among HLA-A antigens, A2 is the most polymorphic antigen and is found at high frequencies in all ethnic groups (Imanish et al., 1992). Identifying the subtypes of HLA-A*02 is of great importance in clinical areas, including organ and bone marrow transplantation (Shintaku et al., 1995), and assessment of disease susceptibility (Thomson, 1995). Especially, HLA-A2 subtypes should be identified for the determination of susceptibility between donor and recipient in unrelated bone marrow transplantation (Anasetti et al., 1995, Davies et al., 1995).

The characterization of the HLA-A2 phenotype has for a long time been relied upon identifying the surface expressed class I molecules through the serological method (Bodmer et al., 1979). However, this method failed to identify all the known variants of many serologically defined A2 specificities. Thus polymorphic epitopes recognized by antibodies may not distinguish between HLA-A2 molecules capable of eliciting very different immune reponses (Saper et al., 1991).

Over the past few years, HLA typing by the DNA method has been used more frequently (Fernandez-Vina et al., 1992; Cereb et al., 1995). The serological typing method can at best identify three A2 specificities. Through DNA typing, we now know of over 20 alleles including 17 major A*02 alleles, all of which encode molecules which have different amino acid sequences and potentially different profiles of immunological function (Fan et al., 1996). The relevance of the polymorphic differences that exist among these variants makes their identification desirable. However, the high degree of sequence homology among the HLA-A*02 alleles and the relative lack of unique polymorphisms have made the identification of individual A*02 alleles difficult.

In this study, HLA-A*02 allelic polymorphisms were determined in HLA-A2 Korean individuals using the nested polymerase chain reaction-sequence specific primers (PCR-SSP) technique.

Materials and Methods

HLA-class I serological typing method was performed as described (Bodmer et al., 1979) in 178 Koreans using commercial typing tray (One lambda Co). Four B-lymphoblastoid cell lines used for verifying primer

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specificity - JY (A*0201), CLA (A*0206), KNE (A*0207) and XL1.ND (A*0210) - were obtained from the 10th International Histocompatibility Workshop. Genomic DNAs were prepared from four B-lymphoblastoid cell lines and lymphocytes from A2 positive Korean individuals by proteinase K digestion and phenol/chloroform extractions (Gustincich et al., 1991).

*HLA-A*02 specific primers*

Amplification of target DNA was achieved by the use of combinations of sequence specific primers as listed in Table 1. The coding primers are specific for sites in exon 2, which encodes for the α1 region of the HLA-A2 molecule, and the non-coding primers are specific for sites in exon 3, which encodes for the α2 region. Amplifications, therefore spanned from exon 2 through to exon 3 including an intron of 240 bp in length. These two exons contain the great majority of HLA-A2 polymorphism (Lawlor et al., 1990). Of the 17 known HLA-A*02 alleles, A*0201 and A*0209 are identical in exons 2 and 3, differing only in exon 4 by a single amino acid (Table 2). Second primer combination of the first round PCR was used to identify the A*0209 type (Table 3).

Table 1. Oligonucleotide primer sequences for HLA-A2 DNA subtyping

Primers	Nucleotide sequence(5'-3')	Length
Coding primers		
AL#3	GGA CGG GGA GAC ACG GAA A	19
AL#13	GTG GAT AGA GCA GGA GGG T	19
AL#14	AAG GCC CAG TCA CAG ACT C	19
AL#22	CCA CTC CAT GAG GTA TTT CTT	21
AL#26	GGA CGG GGA GAC ACG GAA T	19
AL#27	CTC ACT CCA TGA GGT ATT TCT A	22
AL#29	CAG CTC AGA CCA CCA AGC A	19
AL#37	TCC TCG TCC CCA GGC TCT	18
AL#55	GAA GGC CCA CC ACA GAT TG	20
Non coding primers		
AL#H	CCA AGA GCG CAG GTC CTC T	19
AL#N	ACC CCA CGT CGC AGC CAA	18
AL#R	CCT CCA GGT AGG CTC TCT G	19
AL#U	CCT CCA GGT AGG CTC TCC	18
AL#AD	GAG CCA CTC CAC GCA CTC	18
AL#AE	CTC CGC CTC ATG GGC CGT	18
AL#AF	CAC GTC GCA GCC ATA CAT CA	20
AL#AK	TAC TGG TGG TAC CCG CGC	18
AL#AL	CTG GAA GGT TCC ATC CCC TT	20
AL#AO	CTC TCT GCT GCT CCG CCA	18
AL#AW	GTG GCC CCT GGT ACC CGT	18
AL#BF	ACC CCA CGT CGC AGC CAT	18
AL#BG	ACG TCG CAG CCA TAC ATC C	19
AL#BJ	CCG ACC CCA CGT CGC AGG CAC	21
AL#BK	GAG CCC GTC CAC GCA CTC	18
AL#BL	CTC TCT GCT GCT CCG CCT	18
AL#CA	CAT GCT GCA CAT GGC AGG TT	20
AL#CB	CCT CCA GGT AGG CTC TCA	18
AL#SM2	CAT GCT GCA CAT GGC AGG TG	20
Positive internal control primers		
5'PIC#1	ATG ATG TTG ACC TTT CCA GGG	21
3'PIC#AN	ATT CTG TAA CTT TTC ATC AGT TGC	24

Table 2. Comparison of the amino acid sequences of the α1-α3 domains of 17 A*02 alleles

	α-1					α-2						α-3
	9	43	66	73-4	95	97	99	107	149	152	156	236
A*0201	F	Q	K	T H	V	R	Y	W	A	V	L	A
A*0202	-	R	-	- -	L	-	-	-	-	-	W	-
A*0203	-	-	-	- -	-	-	-	-	T	E	W	-
A*0204	-	-	-	- -	-	M	-	-	-	-	-	-
A*0205	Y	R	-	- -	L	-	-	-	-	-	W	-
A*0206	Y	-	-	- -	-	-	-	-	-	-	-	-
A*0207	-	-	-	- -	-	-	C	-	-	-	-	-
A*0208	Y	R	N	- -	L	-	-	-	-	-	W	-
A*0209	-	-	-	- -	-	-	-	-	-	-	-	E
A*0210	Y	-	-	- -	-	-	F	G	-	-	-	-
A*0211	-	-	-	I D	-	-	-	-	-	-	-	-
A*0212	-	-	-	- -	-	-	-	-	-	-	Q	-
A*0213	-	-	-	- -	-	-	-	-	-	E	Q	-
A*0214	Y	R	-	- -	L	-	-	-	-	-	-	-
A*0215	-	-	-	- -	-	-	C	-	-	-	-	-
A*0216	-	-	-	- -	-	-	-	-	-	-	-	-
A*0217	-	-	-	- -	L	M	F	-	-	-	-	-

Differences in the sequences are indicated. Amino acids identical to those in the sequence encoded by A*0201 are indicated by dashes (Saper et al., 1991; Zemmour and Parham., 1992).

Amplification of DNA

The PCR reactions were carried out in 25 μl volumes, containing 17 mM ammonium sulphate, 67 mM Tris HCl (pH 8), 6.7 μM disodium EDTA, 0.0017% BSA, 200 μM of each dNTP, 1.65 mM magnesium chloride, 0.35 μM of each of the two control primer, 0.7 μM of two relevant sequence-specific primers and 100 ng of target DNA. The mixture was spun down prior to adding 0.16 U of *Taq* polymerase. A negative control reaction, containing distilled water instead of DNA was included to test the PCR mixture contamination.

Table 3. Primer mixes, size of specific product, and specificity of the HLA-A2 locus

Primer mix No	Specificity	Size (bp)
First round		
01	A*02	813
02	A*0209	907
03	A*0215	971
04	all A*02 except A*0215	971
Second round		
01	A*0201/0204/0207/0209/0211 A*0215/0216/0217	715
02	A*0202	597
03	A*0203	694
04	A*0204/0209/0217	540
05	A*0202/0205/0214	409
06	A*0205/0208	716
07	A*0207/0215	549
08	A*0208	408
09	A*0206/0210/0214	715
10	A*0210	546
11	A*0211	522
12	A*0212/0213	705
13	A*0203/0213	695
14	A*0216	595
15	A*all A*02 except A*0211	437
16	A*0205/0206/0208/0214	549
17	A*0201/0202/0204/0207/0219 0211/0212/0215/0216/0217	705
18	A*0217	545
19	A*0205/0206/0210	541

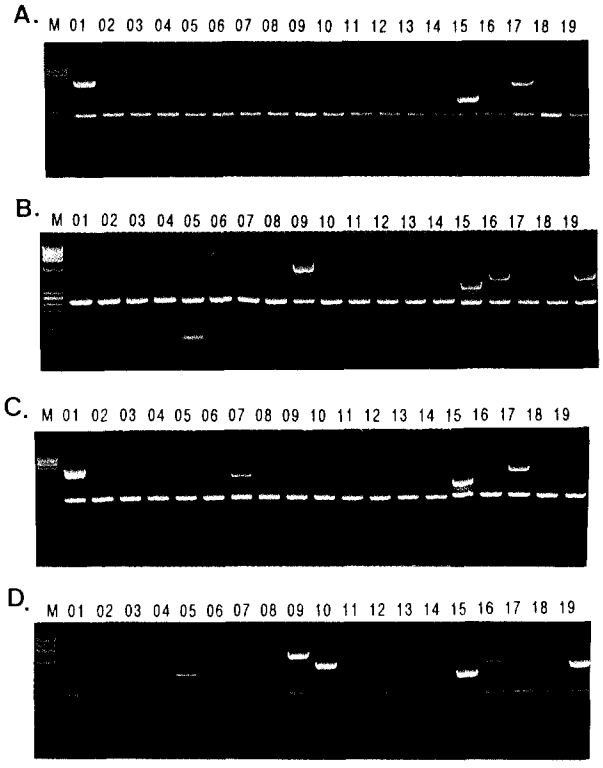

Fig. 1. HLA-A*02 DNA typing in B-lymphoblastoid cell lines using nested PCR-SSP. Amplified DNA bands of 4 cell lines are remarked as follows.

A. A*0201(JY) : 1, 15, 17
B. A*0206(CLA) : 9, 15, 16, 19
C. A*0207(KNE) : 1, 7, 15, 17
D. A*0210(XL1.ND) : 9, 10, 15, 19

01 A*0201/0204/0207/0209/0211 A*0215N/0216/0217
02 A*0202
03 A*0203
04 A*0204/0209/0217
05 A*0202/0205/0214
06 A*0205/0208
07 A*0207/0215N
08 A*0208
09 A*0206/0210/0214
10 A*0210
11 A*0211
12 A*0212/0213
13 A*0203/0213
14 A*0216
15 A*all A*02 except A*0211
16 A*0205/0206/0208/0214
17 A*0201/0202/0204/0207/0219 0211/0212/0215N/0216/0217
18 A*0217
19 A*0205/06/10

The amplification was carried out in an automatic thermal cycler (GeneAmp PCR system 9600: Perkin-Elmer Cetus Inc. Norwalk, USA). The PCR parameters for the 30 cycle, first round amplification consisted of 95℃ for 25 sec, 70℃ for 45 sec, 72℃ for 30 sec. Ten microliters of each PCR reaction were screened on a 2% agarose gel stained with ethidium bromide to check for the A*02 specific amplification. A 1:100 dilution of the A*02 specific PCR product was made, and 2 μl of this was added to a final 13 μl volume of each of the second subtyping reaction. The panel of reactions was then run on a 15 cycle PCR consisting of 95℃ for 25 sec, 65℃ for 45sec, 72℃ for 30 sec. The subtyping reactions were then visualized on a 2% agarose gel containing 0.5 μg/ml ethidium bromide. And the result was interpreted by the presence or absence of the appropriately sized PCR products in each of the panel reactions (Table 3).

Table 4. Distribution of A2 antigen in unrelated Koreans determined by serological typing method (n=178)

HLA-A	Frequency (%)	HLA-A	Frequency (%)
A1	2.1	A29	0.4
A2	27.0	A30	4.2
A3	1.2	A31	7.5
A11	11.7	A32	0.8
A23	-	A33	16.4
A24	20.8	A34	-
A25	-	A36	-
A26	7.1	A43	-
A28	-	ABL	0.8

Results

HLA-A2 is the most frequent HLA-A antigen determined by serological typing method in 178 healthy unrelated Korean individuals. Forty eight individuals (27.0%) were A2 antigen positive. A24 and A33 antigens were also frequently found at 20.8% and 16.4%, respectively, in Korean individuals (Table 4). The results of the four B-lymphoblastoid cells were consistant with the previous report by Krausa et al. (1995) using PCR analysis. Fig. 1 shows the amplified DNA bands of four HLA-A*02 lymphoblastoid cell lines using nested PCR-SSP.

*Distribution of A*02 alleles in Korean*

The A*02 subtypes were analyzed in a total of 53 genes found in 48 A2 Korean individuals using nested PCR-SSP. Of these, five A2 homozygous samples were observed as two A*0207 and one A*0206 homozygotes, and two A*0206/*0207 heterozygotes. Five A*02 variants, A*0201, A*0203, A*0206, A*0207 and A*0210, were the major alleles and the other twelve A*02 alleles were not found in Koreans (Table 5). The most frequent allele found in Koreans was A*0207 (49.0%). A*0206 and A*0201 were also frequently observed at 28.3% and 17.0%, repectively. A*0203 and A*0210 alleles were determined in less than 5% (Table 5).

Discussion

HLA-A2 molecule shows a greater degree of flexibility in accommodating a number of different anchor residues at α1 and α2 regions than in other class I molecules (Falk et al., 1991). The increased prevalence of these amino acid anchor residues in proteins generally make HLA-A2 potentially capable of presenting a large array of peptides. This may also partially explain the prevalence of HLA-A2 in several ethnic groups. The gene frequency of HLA-A2 has been defined by serology in Korean at 27% (Table 4), in British population 24% (Krausa et al., 1995), in Japanese at 24.4%, in Han Chinese at 35.7%, in Singapore Chinese at 37%, in Mongolian

at 23.1%, and in Black population at 18% (Imanishi et al., 1992).

Table 2 illustrates the polymorphic amino acid residues which characterize the gene products of the 17 A*02 alleles defined (Saper et al., 1991; Zemmour and Parham., 1992). The polymorphism amongst the A*02 variants occurs mainly as a result of different combinations of sequence motifs shared with other A*02 and class I alleles at hypervariable regions within the gene (Kubo et al., 1994). The possibility of different combinations of A*02 polymorphic motifs generates a potentially large number of A*02 subtypes in excess of alleles presently identified. All the polymorphisms which distinguish the different HLA-A2 molecules potentially have functional relevance in terms of the T cell response (McMichael et al., 1988; Moss et al., 1991; Goulmy et al., 1995). Discrimination of these A*02 subtypes at the molecular level has a significant impact on the clinical area.

Recently, several molecular typing methods, such as PCR-SSOP (sequence specific oligonucleotide probe) (Schart et al., 1991), PCR-RFLP (restriction fragment length polymorphism) (Lee, 1995; Lee and Park, 1995), PCR-SSP (sequence specific primers) (Lee et al., 1996) and sequence based typing method (Rozemuller et al., 1996), have been used for the determination of HLA genes. PCR-SSOP provides one of the best direct definitions of HLA polymorphism. However, a precise determination of the official A*02 alleles necessitates a high number of SSO probes, each with specific washing conditions (Schart et al., 1991). The PCR-RFLP method saves time in HLA typing and enzyme digestion is completed within 3 hrs or less. However allele determination is difficult when restriction sites were not found in A*02 alleles (Lee, 1995). PCR-SSP has been applied to the determination of HLA-class I genes, including HLA-A (Browning et al., 1993; Krausa et al., 1993), HLA-B (Sadler et al., 1994; Bunce et al., 1995), and HLA-C (Bunce and Welsh, 1994). The principle of PCR-SSP is that each group of alleles or individual allele making up a serological specificity is amplified by a primer pair matched exactly to that group. PCR-SSP is a simple and rapid technique and suitable for the low resolution DNA typing and for samples of small volume (Krausa et al., 1993). These DNA typing methods should be chosen according to the sample size and the purpose of experiment.

In the present study, nested PCR-SSP was used for the determination of HLA-A2 allelic polymorphism. The primer sequences were chosen in polymorphic exons 2 and 3 including intron to discriminate A*02 alleles. These two exons code for the α1 and α2 domains of the A2 molecule, which form the α helices and β-pleated sheet which surround the peptide binding groove (Bjorkman et al., 1987). The nested PCR-SSP was performed by the following two steps. The first round PCR reaction which excludes all non A*02 class I alleles, is useful as a powerful approach in differentiating between this group of highly similar alleles because only a few A*02 alleles contain unique sequence motifs. The second PCR was performed with the diluted PCR product from the first round HLA-A*02 mix as DNA templates (Table 3). In the A*02 subtyping previously reported by Krausa et al. (1995), one and 14 primer pairs were used for the first and second PCR, respectively, for the determination of 14 A*02 subtypes. In this study, we modified the previous method by Krausa et al. (1995) to identify three additional A*02 alleles, A*0215, A*0216 and A*0217. Seventeen A*02 specific alleles could be discriminated using modified nested PCR-SSP method. Four and nineteen A*02 allele specific PCR primer pairs were used for the first and second round PCR, respectively. The details of this panel of reactions are given in Table 3. Amplification of genomic DNA yielded A*02 specific PCR products ranging in size from 408 to 971bp (Table 3). Each reaction was controlled internally with primers that amplify a 330 bp region of the human β-2 microglobulin gene to ensure the PCR is capable of functioning. Sequencing analysis should be performed to confirm the A*02 allele which was not defined accurately by using this PCR-SSP method.

Evidence from allelic typing in unrelated bone marrow transplant screening showed a significant incidence of mismatch for A*02 alleles between potential donors and recipients which had been typed by serology (Anasetti et al., 1995). For this reason, HLA-A2 population studies which can identify allelic frequencies, may prove essential for recognizing

Table 5. Distribution of A*02 alleles in Korean and East-Asian ethnic groups and Caucasian

HLA-A*02	Korean[d] n=53	Chinese[b] Dai n=46	Chinese[a] Singapore n=66	Chinese[c] Thai n=52	Chinese[c] Man n=100	Japanese[c] n=46	Caucasian[b] n=96
A*0201	17.0	2.9	23.0	20.0	48.6	45.2	97.4
A*0202	-	-	-	-	-	-	-
A*0203	1.9	28.6	23.0	40.0	11.4	-	-
A*0204	-	2.9	-	-	-	-	-
A*0205	-	-	-	-	1.4	-	2.6
A*0206	28.3	2.9	8.0	15.0	22.9	35.4	-
A*0207	49.0	68.6	45.0	37.5	15.7	16.4	-
A*0208	-	-	-	-	-	-	-
A*0209	-	-	-	-	-	-	-
A*0210	3.8	-	2.0	2.5	-	3.0	-
A*0211	-	-	-	2.5	-	-	-
A*0212	-	-	-	-	-	-	-
A*0213	-	-	-	-	-	-	-
A*0214	-	-	-	-	-	-	-
A*0215	-	-	-	-	-	-	-
A*0216	-	-	-	-	-	-	-
A*0217	-	-	-	-	-	-	-

[a] reported by Krausa et al., 1995
[b] reported by Fan et al., 1996
[c] reported by Ishikawa et al., 1996
[d] determined by this study

which variants to match for in a given ethnic group. In order to assess the A*02 subtype frequencies in the population of Korea, a total of 53 genes found in 48 A2 healthy unrelated individuals have been analyzed using nested PCR-SSP. This study indicated that A*0207 was by far the dominant A*02 subtype in Korean (Table 5). Moreover the results were compared to those on East-Asian ethnic groups, including Japanese and several Chinese. Five A*02 alleles, A*0201, A*0203, A*0206, A*0207 and A*0210 were common in Asian ethnic groups. A*0207 was the predominant in Korean (49.0%), Dai (68.6%) and Singapore Chinese (45.0%) whereas it was not common in Japanese (16.4%), Han Chinese (19.5%), and absent in the Caucasian population (0%). In contrast, A*0201 represented nearly all the HLA-A*02 alleles in Caucasian (97.4%) and it was also the most prevalent allele in both Japanese (45.2%) and Manchurian (48.6%) (Fan et al., 1996; Ishikawa et al., 1996) (Table 5).

Our results indicate the importance of ethnic origin in terms of the expected HLA-A*02 allelic profile. Moreover, this study shows that molecular typing will redefine HLA-A*02 subtype frequencies and may prove useful for optimal matching of HLA-A*02 donor- recepient pairs in unrelated bone marrow transplantation.

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