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# Molecular Analysis of HLA-C Using Polymerase Chain Reaction-Sequence Specific Primers

**Kyung-Ok Lee*, Sung-Hoi Hong, Min-Jung Kim[1], Taek-Kyu Park[1], Yoon-Jung Kim and Kyu-Pum Lee**

*Seoul Clinical Laboratories, Seoul Medical Science Institute, Seoul 140-230*
*[1]Department of Chemistry, College of Science, Kon-Kuk University, Seoul 133-701, Korea*
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**Abstract :** Of all HLA class I molecules, HLA-C gene products are most poorly understood because they express at a low level on the cell surface compared to HLA-A and -B. In order to identify serologically detectable and undetectable HLA-C antigens, we have established a DNA-based tissue typing method for the HLA-C locus by PCR-SSP (polymerase chain reaction-sequence specific primers). Genomic DNA prepared from lymphoblastoid 21 B-cell lines and 120 Korean individuals by proteinase K digestion and phenol/chloroform extractions have been typed by PCR-SSP (23 primer mixes were used). The PCR-SSP results of control cell lines were discrepant from serology in 1 case among 21 cases: Cw6 which was negative by serology but positive by PCR-SSP (cell line: MANIKA). Twenty four HLA-Cw "blank" antigens among fifty Korean individuals were completely determined by PCR-SSP DNA typing. HLA-Cw*0101 (15.3%), Cw*1401 (12.3%) and Cw*0701 (11.7%) alleles were frequently found in 120 Korean individual samples. In conclusion, the high level of discrimination for HLA-C alleles may prove useful and informative in the study of transplant survival, and identify the importance of allelic differences, not readily detectable by serology, on host and donor compatibility.

**Key words :** DNA typing, HLA-C, polymerase chain reaction-sequence specific primers.

The molecular analysis and characterization of HLA-C allelic polymorphism has been impeded by lack of serological reagents which identify individual HLA-C antigens. At present, 39 HLA-C alleles have been officially designated (Little *et al.*, 1996). The eight specificities Cw1 to Cw8 comprise a total of 19 alleles. In addition 20 leles have no serological counterparts, and they are typed as "blanks" (Levine and Yang, 1994). Complement-mediated microcytotoxicity (serology) using human alloantisera is the most widely used method of typing the HLA class I antigens including HLA-C. This method requires a large panel of human alloantisera, each with single or multiple specificities for HLA antigens. Sources of reagents are limited and not generally renewable, and usually several antibodies are required to assign each antigen due to the complex patterns of serological cross reactivity between individual HLA determinants (Fernandez *et al.*, 1992). Serological typing of HLA-Cw antigens is more difficult than other class I molecules such as HLA-A and -B. HLA-Cw blank antigens are still present at the gene frequency of 30~50% in all human races (Aizawa, 1986). Since only detailed analysis of HLA-C alleles will help to assess the influence of HLA-C on the allogeneic immune response, it seems essential to characterise HLA-C alleles at the nucleotide level. The development of HLA-C DNA typing has been made difficult by the complexity of nucleotide substitutions in that every allele is a combination of sequence motifs shared with other alleles, not only of the same gene but also at other class I loci (Schwartz, 1985; Lawlor *et al.*, 1990). The serological typing method has been replaced by genetic typing using PCR (Polymerase Chain Reaction) technology in the elucidation of HLA gene polymorphism. Such approaches have already been successful with HLA class II genes (Lee *et al.*, 1996). It is only recently that a sufficient number of HLA class I DNA sequences have been available to enable similar development of class I DNA typing (Yoshida *et al.*, 1992). Molecular typing of the HLA-C gene has been reported, such as PCR-SSOP (Sequence Specific Oligonucleotide Probe) (Levine and Yang, 1994; Kennedy *et al.*, 1995) and PCR-SSP (Bunce and Welsh, 1994; Ando *et al.*, 1996;

*To whom correspondence should be addressed.
Tel : 82-2-7906-500, Fax : 82-2-7906-509, 517.

Little *et al.*, 1996). In this study, HLA-C DNA typing using PCR-SSP was developed and compared with the serological typing method. Moreover, HLA-C allelic polymorphism in Korean was determined.

## Materials and Methods

### Materials

Twenty one B-lymphoblastoid homozygous cell lines used for verifying primer specificity were obtained from the Xth International Histocompatibility Workshop.

For the determination of HLA-C allelic polymorphism, 120 Korean individual samples were used. Serological typing was performed on peripheral blood lymphocytes as described (Marsh *et al.*, 1990).

### HLA-C locus specific primers

HLA-C locus specific primer sequences were derived from HLA class I sequences (Zemmour and Parham, 1992). Amplification primers were synthesized using a 392 DNA/RNA synthesizer and purified by an oligonucleotide purification cartridge (Perkin Elmer Inc. Norwalk, USA). The specificity of each individual primer synthesis was tested against 21 B-lymphoblastoid homozygous cell lines. The nucleotide sequences of the 26 primers are shown in Table 1. Primers were designed for the PCR cycle profile and the compositions of the 23 PCR reaction mixtures were adjusted to obtain highly specific and sensitive amplifications for the assignment of all phenotypically expressed HLA-C polymorphism (Table 2). Amplification control primers giving rise to a 796 base pair fragment from the third intron of HLA-DRB1 were included in every PCR reaction. When there is an absence of a specific band, the presence of a positive internal control band verifies the success of the PCR reaction, thus avoiding assignment of false negatives. Each typing was run with a negative control reaction containing distilled water in place of DNA.

**Table 1.** Primer sequence for HLA-C DNA typing using PCR-SSP

| Primers | Nucleotide sequence (5'-3') | | | | | | | Length |
|---|---|---|---|---|---|---|---|---|
| Cw#27 | CCG | AGT | GAA | CCT | GCG | GAA | A | 19 |
| Cw#30 | TAC | TAC | AAC | CAG | AGC | GAG | GA | 20 |
| Cw#31 | CAC | AGA | CTG | ACC | GAG | TGA | G | 19 |
| Cw#32 | AGT | CCA | AGA | GGG | GAG | CCG | | 18 |
| Cw#34 | CCA | CTC | CAT | GAG | GTA | TTT | CTC | 21 |
| Cw#130 | CCG | CGG | GTA | TGA | CCA | GTC | | 18 |
| Cw#136 | TCC | GCG | GGT | ATG | ACC | AGT | A | 19 |
| Cw#159 | TAC | AAC | CAG | AGC | GAG | GCC | A | 19 |
| Cw#160 | ACA | ACC | AGA | GCG | AGG | CCG | | 18 |
| Cw#165 | ACG | ACA | CGC | AGT | TCG | TGC | A | 19 |
| Cw#35 | TCT | TCT | CCA | GAA | GGC | ACC | AT | 20 |
| Cw#40 | CCT | CCA | GT | AGG | CTC | TCC | A | 19 |
| Cw#41 | CAG | CCC | CTC | GTG | CTG | CAT | | 18 |
| Cw#42 | CGC | GCG | CTG | CAG | CGT | CTT | | 18 |
| Cw#45 | CCT | CCA | GGT | AGG | CTC | TCA | G | 19 |
| Cw#126 | TGA | GCC | GCC | GTG | TCC | GCA | | 18 |
| Cw#127 | GGT | CGC | AGC | CAT | ACA | TCC | A | 19 |
| Cw#127.1 | GGT | CGC | AGC | CAA | ACA | TCC | A | 19 |
| Cw#135.1 | AGC | GTC | TCC | TTC | CCA | TTC | TT | 20 |
| Cw#143 | GCC | CCA | GGT | CGC | AGC | CAA | | 18 |
| Cw#145 | GAG | CCA | CTC | CAC | GCA | CTC | | 18 |
| Cw#146 | CCC | TCC | AGG | TAG | GCT | CTC | T | 19 |
| Cw#147 | TCG | TAG | GCT | AAC | TGG | TCA | TG | 20 |
| Cw#157 | CCG | CCG | TGT | CCG | CGG | CA | | 17 |
| Cw#166 | GCG | CAG | GTT | CCG | CAG | GC | | 17 |
| Cw#184 | GCC | ACG | GGC | CGC | CTC | CA | | 17 |
| Positive internal control primers | | | | | | | | |
| 5'C5 | TGC | CAA | GTG | GAG | CAC | CCA | A | 19 |
| 3'C3 | GCA | TCT | TGC | TCT | GTG | CAG | AT | 20 |

### DNA extraction and amplification

Genomic DNA was prepared by proteinase K digestion and phenol/chloroform extractions (Gustincich *et al.*,

**Table 2.** Primer mixes, size of specific product and specificity of HLA-C locus

| Primer mix No. | Specificity | Size bp | Specific primer 1 | Specific primer 2 |
|---|---|---|---|---|
| 01C | Cw*01 | 1026 | Cw#136 | Cw#35 |
| 02C | Cw*02/1701 | 521 | Cw#27 | Cw#145 |
| 03C | Cw*03 | 563 | Cw#31 | Cw#135.1 |
| 04C | Cw*04 | 330 | Cw#27 | Cw#143 |
| 05C | Cw*0501 | 563 | Cw#27 | Cw#42 |
| 06C | Cw*0602 | 304 | Cw#30 | Cw#127 |
| 07C | Cw*0701/0702/0703 | 1062 | Cw#130 | Cw#41 |
| 08C | Cw*08 | 162 | Cw#165 | Cw#166 |
| 09C | Cw*0303 | 530 | Cw#159 | Cw#135.1 |
| 10C | Cw*0302/304 | 529 | Cw#160 | Cw#135.1 |
| 11C | Cw*0302 | 206 | Cw#130 | Cw#135.1 |
| 12C | Cw*1201/1202/1301 | 449 | Cw#31 | Cw#126 |
| 13C | Cw*1201/1202 | 537 | Cw#32 | Cw#126 |
| 14C | Cw*1203 | 453 | Cw#31 | Cw#157 |
| 15C | Cw*14 | 541 | Cw#34 | Cw#127.1 |
| 16C | Cw*15 | 373 | Cw#27 | Cw#147 |
| 17C | Cw*15/1701 | 500 | Cw#27 | Cw#45 |
| 18C | Cw*1601 | 512 | Cw#31 | Cw#146 |
| 19C | Cw*1602 | 512 | Cw#27 | Cw#146 |
| 20C | Cw*0602/1602 | 324 | Cw#27 | Cw#127 |
| 21C | Cw*02/0602 | 501 | Cw#27 | Cw#40 |
| 22C | Cw*0704 | 563 | Cw#30 | Cw#42 |
| 23C | Cw*1701 | 475 | Cw#27 | Cw#184 |

1991). The PCR reactions were carried out in 13 μl volumes, containing 17 mM ammonium sulphate, 67 mM Tris HCl (pH 8), 6.7 μM disodium EDTA, 0.017% BSA, 200 μM of each dNTP, 1.5 mM magnesium chloride, 0.07 μM of each of the control primer, 0.7 μM of relevant sequence specific primers and 100 ng of target DNA. The reaction mixture was heated to 95°C for 5 min to denature the DNA and cooled to room temperature. The condensation was spun down prior to adding 0.16 U of Taq polymerase. The amplifications were carried out in an automatic thermal cycler (GeneAmp PCR system 9600; Perkin-Elmer Cetus Inc. Norwalk, USA.) for over 30 cycles. The PCR cycle parameter was 95°C for 25 sec, 70°C for 45 sec and 72°C for 30 sec. Ten microliters of each PCR reaction was run on a 2% agarose gel containing 0.5 μg/ml ethidium bromide. The gels were run for 15 min at 15 V/cm in 0.5×TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8.0). Gels were examined under UV illumination and documented by photography. The amplified DNA bands were compared with the *Phi*X/*Hae*III molecular weight marker.

## Results

The PCR-SSP results of control cell lines were discordant with serological data in 1 case among 21 cases tested. Cell line MANIKA tyed for Cw*06 by DNA typing but Cw6 antigen was not identified by serological typing (Table 3). Five HLA-Cw "blank" alleles of control cells were typed as Cw*12 (9062, WDW; 9008, D 0208910), Cw*14 (9070, LUY), Cw*16 (9051, PIOUT) and Cw*17 (9043, BM21) (Table 3). The results of other control cells were consistant with the previous reports typed by PCR analysis (Table 3).

### Comparison of HLA-C DNA typing with serological typing

Fifty Korean individual samples were typed by using both serological typing and DNA typing. Two Cw3/Cw7

**Table 3.** The correlation between HLA-C DNA typing and serological typing in 21 B-lymphoblastoid cell lines

| WSNb[a] | Identity | HLA-C antigen by serology[b] | HLA-C allele by PCR-SSP[c] | HLA-C allele by PCR-SSP[d] | HLA-C allele by PCR-SSOP[e] | HLA-C allele by PCR-SSOP[f] |
|---|---|---|---|---|---|---|
| 9102 | ARBO | Cw6 | Cw*06 | – | Cw*06 | Cw*0601 |
| 9068 | BM9 | Cw4 | Cw*04 | – | Cw*04 | Cw*0401 |
| 9033 | BM14 | Cw7 | Cw*07 | – | – | Cw*0701 |
| 9038 | BM16 | Cw7 | Cw*07 | – | – | Cw*0701 |
| 9043 | BM21 | Cw– | Cw*17 | Cw*1701 | Cw*1701 | Cw*1701 |
| 9007 | DEM | Cw6 | Cw*06 | Cw*06 | Cw*06 | Cw*0601 |
| 9008 | D0208910 | Cw– | Cw*12 | – | Cw*1203 | Cw*1203 |
| 9054 | EK | Cw5 | Cw*05 | Cw*05 | Cw*05 | Cw*05 |
| 9055 | H0301 | Cw8 | Cw*08 | – | Cw*0802 | Cw*0802 |
| 9030 | JVM | Cw8 | Cw*08 | – | Cw*0801 | – |
| 9083 | LD2B | Cw7 | Cw*07 | – | Cw*07 | Cw*07 |
| 9070 | LUY | Cw– | Cw*14 | Cw*14 | Cw*14 | Cw*14 |
| 9002 | MZ070782 | Cw8 | Cw*08 | Cw*8 | – | Cw*8 |
| 9051 | PIOUT | Cw– | Cw*16 | Cw*1601 | Cw*1601 | Cw*1601 |
| 9047 | PLH | Cw6 | Cw*06 | – | Cw*1601 | Cw*0601 |
| 9020 | QBL | Cw5 | Cw*05 | Cw*05 | Cw*05 | Cw*05 |
| 9076 | R7526 | Cw1 | Cw*01 | Cw*01 | – | – |
| 9062 | WDV | Cw– | Cw*12 | Cw*1203 | Cw*1203 | Cw*12 |
| 9106 | MANIKA | Cw– | Cw*06 | Cw*0601 | – | Cw*06 |
| 9006 | WT100 | Cw4 | Cw*04 | – | – | Cw*0401 |
| 9023 | VAVY | Cw7 | Cw*07 | Cw*07 | Cw*0701 | Cw*0701 |

– : Not tested.
[a] Workshop Number (10th International HLA Workshop).
[b] Analysis by serological typing in 10th International Workshop.
[c] Analysed by authors using PCR-SSP DNA typing.
[d] Analysed by Bunce and Welsh (1994) using PCR-SSP DNA typing.
[e] Analysed by Kenney *et al.* (1995) using PCR-SSOP DNA typing.
[f] Analysed by Levine and Yang (1994) using PCR-SSOP DNA typing.

cases by serological typing were identified by Cw*07/Cw*10 (S7) and Cw*07/Cw*09 (S22) types with PCR-SSP DNA typing. Twenty four HLA-C blank alleles among 50 individual samples were detectable using DNA typing as Cw*01 (2 cases), Cw*03 (1 case), Cw*05 (2 cases), Cw*08 (5 cases), Cw*12 (1 case) C*14 (9 cases) and Cw*15 (4 cases), respectively (Table 4).

**Table 4.** The correlation between HLA-C DNA typing and serological typing in 50 Korean individuals

| Sample No. | Cw antigen by serology | Cw allele by PCR-SSP | Sample No | Cw antigen by serology | Cw allele by PCR-SSP |
|---|---|---|---|---|---|
| S1 | Cw3, - | Cw*01/*03 | S26 | Cw-, - | Cw*05/*05 |
| S2 | Cw1, 3 | Cw*01/*03 | S27 | Cw-, - | Cw*14/*15 |
| S3 | Cw7, - | Cw*07/*08 | S28 | Cw4, 5 | Cw*04/*05 |
| S4 | Cw3, 7 | Cw*03/*07 | S29 | Cw6, - | Cw*06/*14 |
| S5 | Cw1, 3 | Cw*01/*03 | S30 | Cw6, - | Cw*06/*14 |
| S6 | Cw1, - | Cw*01/*14 | S31 | Cw1, 3 | Cw*01/*03 |
| S7 | Cw3, 7 | Cw*07/*10 | S32 | Cw3, 7 | Cw*03/*07 |
| S8 | Cw3, 7 | Cw*03/*07 | S33 | Cw3, - | Cw*03/*15 |
| S9 | Cw3, - | Cw*03/*08 | S34 | Cw6, 7 | Cw*06/*07 |
| S10 | Cw3, 6 | Cw*03/*06 | S35 | Cw3, 6 | Cw*03/*06 |
| S11 | Cw6, 7 | Cw*06/*07 | S36 | Cw3, - | Cw*03/*12 |
| S12 | Cw1, 6 | Cw*01/*06 | S37 | Cw4, - | Cw*04/*14 |
| S13 | Cw1, 6 | Cw*01/*06 | S38 | Cw1, 3 | Cw*01/*03 |
| S14 | Cw1, 6 | Cw*01/*06 | S39 | Cw3, 4 | Cw*03/*04 |
| S15 | Cw3, 7 | Cw*03/*07 | S40 | Cw7, - | Cw*07/*14 |
| S16 | Cw6, - | Cw*06/*14 | S41 | Cw3, - | Cw*03/*08 |
| S17 | Cw1, 3 | Cw*01/*03 | S42 | Cw1, - | Cw*01/*08 |
| S18 | Cw3, 7 | Cw*03/*07 | S43 | Cw-, - | Cw*01/*01 |
| S19 | Cw4, - | Cw*04/*15 | S44 | Cw1, - | Cw*01/*14 |
| S20 | Cw4, - | Cw*04/*08 | S45 | Cw3, 7 | Cw*03/*07 |
| S21 | Cw1, 1 | Cw*01/*01 | S46 | Cw3, 5 | Cw*03/*05 |
| S22 | Cw3, 7 | Cw*07/*09 | S47 | Cw-, - | Cw*14/*15 |
| S23 | Cw1, 4 | Cw*01/*04 | S48 | Cw1, 3 | Cw*01/*03 |
| S24 | Cw1, 1 | Cw*01/*01 | S49 | Cw1, 3 | Cw*01/*03 |
| S25 | Cw1, 1 | Cw*01/*01 | S50 | Cw3, 7 | Cw*03/*07 |

## Distribution of HLA-C alleles in Koreans

The most frequently observed HLA-C allele was Cw*0101 (15.3%) in 120 Korean individuals. Cw*1401

**Table 5.** Allelic distributions of HLA-C gene

| HLA-Cw allele | Frequency[a] in Korean (%) (n=120) | Frequency[b] in Japanese (%) (n=225) |
|---|---|---|
| Cw*0101 | 15.3 | 18.5 |
| Cw*0201 | 0.8 | 0 |
| Cw*0301 | 6.3 | 0.4 |
| Cw*0302 | 8.3 | 0 |
| Cw*0303 | 6.5 | 10.8 |
| Cw*0304 | 4.6 | 10.8 |
| Cw*0401 | 5.4 | 4.4 |
| Cw*0501 | 2.9 | 0.4 |
| Cw*0601 | 7.9 | 0.2 |
| Cw*0701 | 11.7 | 13.7 |
| Cw*0801 | 4.6 | 12.6 |
| Cw*1201 | 7.7 | 12.2 |
| Cw*1203 | 0 | 0 |
| Cw*1301 | 0 | 0 |
| Cw*1401 | 12.3 | 11.6 |
| Cw*1501 | 2.3 | 3.6 |
| Cw*1601 | 1.2 | 0 |
| Cw*1602 | 1.2 | 0 |
| Cw*1701 | 0 | 0 |

[a] Analysed by authors using PCR-SSP.
[b] Analysed by Ando *et al.* (1996) using PCR-SSP in Japanese.

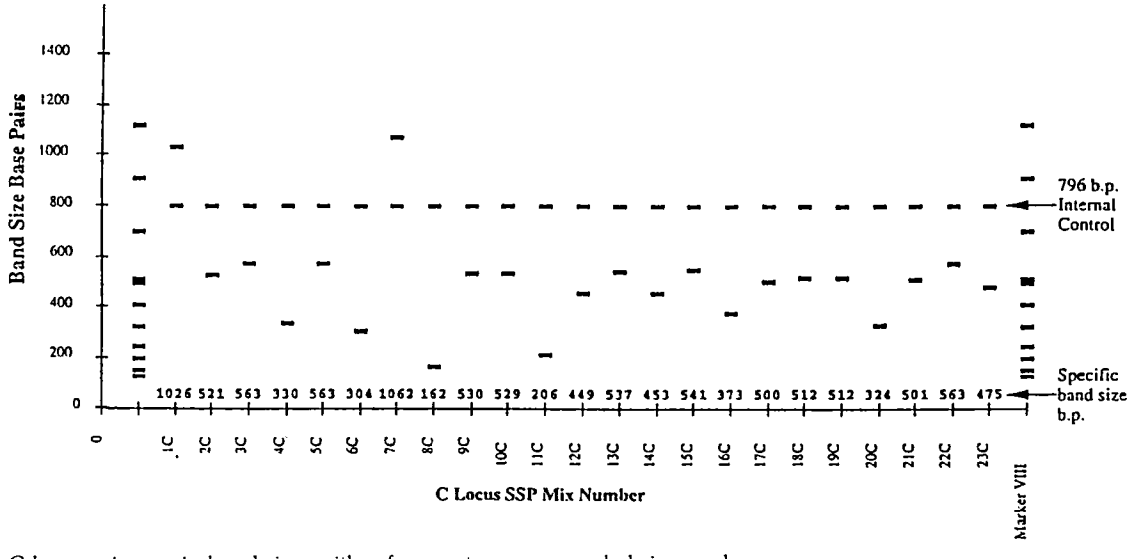


**Fig. 1.** HLA-C locus primer mix band sizes with reference to recommended size marker.

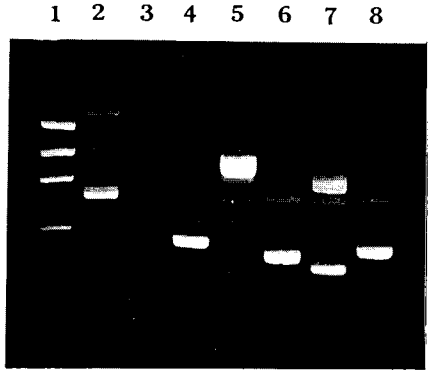


**Fig. 2.** HLA-C locus DNA typing of B lymphoblastoid cell lines by PCR-SSP.

| | | | |
|---|---|---|---|
| lane 1 | : Molecular Weight Size Marker (ϕX174/*Hae*III) | | |
| lane 2 | : Internal control (796 bp) | | |
| lane 3 | : Negative control | | |
| lane 4 | : DKB | ; HLA-Cw*03 | 563 bp |
| lane 5 | : VAVY | ; HLA-Cw*07 | 1062 bp |
| lane 6 | : WT24 | ; HLA-Cw*02 | 521 bp |
| lane 7 | : RSH | ; HLA-Cw*17 | 475 bp |
| lane 8 | : LUY | ; HLA-Cw*14 | 541 bp |

(12.3%) and Cw*0701 (11.7%) were also frequently determined (Table 5). Fig. 1 illustrates the relative sizes of the PCR products obtained in each of the 23 PCR reactions of the HLA-C PCR-SSP typing panel. Fig. 2 and 3 show the amplified PCR products of five B-lymphoblastoid homozygous cell lines and Cw*01/*08 heterozygous Korean individual samples respectively.

## Discussion

The role of HLA-C in transplantation, immunoregulation and disease susceptibility has been largely unknown. However, recently several functions have been elucidated which suggest that HLA-C does have a clinical and immunoregulatory role (Bunce and Welsh, 1994) In transplantation, HLA-C can act as a target for hyperacute rejection (Shimizu and DeMars, 1989; Petersdorf *et al.*, 1994) and cytotoxic HLA-C specific lymphocytes can be generated in vitro (Grunnet *et al.*, 1976) and may be responsible in vivo for graft rejection (Bonneville *et al.*, 1988; Bann *et al.*, 1992). Various HLA-C disease associations have been reported which lend credence to an immunological role for HLA-C (D'Amaro *et al.*, 1984; Green *et al.*, 1988; Sakkas *et al.*, 1991; Levine and Yang, 1994). The serological method for HLA-C is generally quick and reliable; however, approximately 20% of Caucasians and up to 50% of Japanese have only one detectable HLA-C allele (Aizawa, 1986). The reason for HLA-C alleles being undetectable by serology is unclear. Possible ex-

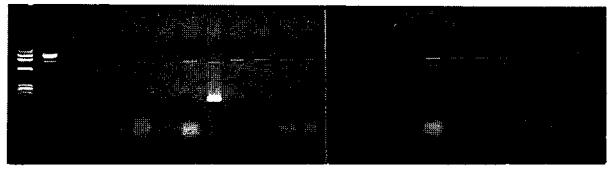

**Fig. 3.** Example of HLA-C PCR SSP typing.

Sample No. 42
Serological type : HLA-Cw,1,-
PCR-SSP type : HLA-CW*01/CW*08
Positive lanes : 1, 8
All typings are loaded in the following order, left to right.

| | | |
|---|---|---|
| 01 Cw*01 | 09 Cw*0303 | 17 Cw*15/1701 |
| 02 Cw*02/1701 | 10 Cw*0302/304 | 18 Cw*1601 |
| 03 Cw*03 | 11 Cw*302 | 19 Cw*1602 |
| 04 Cw*04 | 12 Cw*1201/1202/1301 | 20 Cw*0602/1602 |
| 05 Cw*0501 | 13 Cw*1201/1202 | 21 Cw*02/0602 |
| 06 Cw*0602 | 14 Cw*1203 | 22 Cw*0704 |
| 07 Cw*0701/0702/0703 | 15 Cw*14 | 23 Cw*1701 |
| 08 Cw*08 | 16 Cw*15 | |

planations include chromosomal deletion, transcription errors, lack of low surface expression and a lack of suitable serological reagents, etc. (Strachan *et al.*, 1986; Tibensky *et al.*, 1988; Ando *et al.*, 1996). The most likely cause of serologically undefined HLA-C alleles is the lack of suitable antisera coupled with low cell surface expression. Despite similar messenger RNA levels, HLA-C antigens are expressed on cell surfaces at approximately 10% of the level of either HLA-A or HLA-B (Gussow *et al.*, 1987). This may be due to inefficient assembly of HLA-C molecules with 2-microglobulin (Neefjes and Ploegh, 1988), or conserved features in the 1 domain may lead to HLA-C selectively binding a restricted set of peptides, which in turn give inefficient assembly and hence lower cell surface expression (Zemmour and Parham, 1992).

Routine HLA class I typing in most laboratories is still performed by the serological method. The advancement of DNA sequencing techniques has shown that polymorphism of the HLA gene is much greater than assessed by serology (Erlich and Bugawan, 1989). Recently, a DNA-based typing method using PCR-SSP has been sucessfully applied to the HLA class I region, including typing of the HLA-A (Browning *et al.*, 1993), HLA-B (Sadler *et al.*, 1994; Bunce *et al.*, 1995) and HLA-C (Bunce and Welsh, 1994) genes as well as class II genes such as the HLA-DRB (Lee *et al.*, 1996) gene. In this study, a rapid and reproducible HLA-C DNA typing method using the PCR-SSP technique was developed. The HLA-C DNA typing panel was designed to cover specifically all known serologically defined antigens in 23 separated allele or group specific reac-

tions as described in Table 2. Amplification of genomic DNA yielded HLA-C locus specific PCR products ranging in size from 162 to 1062 bp. with a positive internal control product of 796 bp (Fig. 1).

The PCR-SSP results of control cell lines were well correlated with previous reports (Bunce and Welsh. 1994: Levine and Yang. 1994: Kenney *et al*.. 1995). Serologically undefined 24 Cw "blank" samples could be identified by PCR-SSP DNA typing (Table 4). Two samples (S7. S22) by DNA typing were discrepant from the serological data. Two Cw3/Cw7 types were identified by Cw*07/Cw*10 (S7) and Cw*07/Cw*09 (S22) types with DNA typing. Cw*09 and Cw*10 alleles were the split of the Cw3 antigen. Therefore. the result of DNA typing. Cw*09 and Cw*10 were the same as serological type Cw3 (Table 4). The Cw*0101 (24.2%) allele was most frequently observed and Cw*0701 (11.7%) and Cw*1401 (16.3%) alleles were also frequently found in Koreans (Table 5). These data were compared with those on Japanese. since the distribution of HLA-C alleles in Koreans has not been reported. Frequently observed HLA-C alleles in Japanese-Cw*0101 (18.5%). Cw*0701 (13.7%) and Cw*1401 (11.6%)-were the same as in Koreans. The frequencies of Cw*0303. Cw*0304. Cw*0801 and Cw*1201 alleles in Japanese were higher than that of Koreans. whereas Cw*0301 and Cw*0601 alleles were more frequent in Koreans (Table 5). Three HLA-C alleles (Cw*1203. Cw*1301. and Cw*1701) were not found in Koreans (Table 5).

The HLA-C DNA typing has several advantages over serological typing. In practical terms. the method uses renewable reagents and standard laboratory equipment. widening the scope of tissue typing for clinical or research purposes (Cereb *et al*.. 1995). Since the method is based on genomic sequences. the typing obtained is definitive (within the confines of known HLA sequences). Furthermore. this molecular typing system does not require viable cells. and can be used to type material from cells that cannot be used for tissue typing by conventional means (*eg*.. epithelial cells and cells with low or undetectable HLA expression) (Krausa *et al*.. 1993).

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