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Serologic Heterogeneity of HLA-A24 Correlates with Allelic Types in the Korean Population

HLA-A24 is the second most frequently expressed HLA-A type in Koreans (GF 22.8%). Four different serologic reaction patterns were observed in Korean A24 positive samples using a commercial serologic typing kit. To clarify the nature of serologic heterogeneity, thirteen A24 positive DNA samples representing the four different serologic reaction patterns were subjected to DNA sequencing analysis of the amplified HLA-A genes from each sample. Four A*24 alleles (A*2402101, A*2403, A*2408, and A*2421) were associated with the four unique serologic reaction patterns. During this study, a novel allele, A*2421, was characterized. The new sequence is similar to A*2402101, differing at codon 127 (AAA→AAC; K→N). By comparing putative amino acid sequences and serologic reaction patterns of A*24 allelic products identified in this study, several crucial sites for A24- and A9-specific antibody binding were predicted: 127K for A24 antibody binding, and 62E-65G and 166D-167G for A9 antibody binding. This information will be helpful for accurately assigning HLA-A24 types by serology and for predicting serologic types of new alleles.

Key Words: *HLA-A24; Serology; Population Characteristics; Heterogeneity; Epitope; Novel Allele; Korean*

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INTRODUCTION

A24 is one of the most frequent HLA-A types in human populations. Twenty alleles (A*2402101-A*2420) belonging to the A24 cluster have been identified thus far (1) and three of them (A*2402102L, A*2409N, A*2411N) have known defects in their normal expression on cell surfaces (2).

HLA class I typing has mostly been performed by serology; however, serology is being replaced by DNA typing for improved accuracy and resolution of the typing results as well as the potential for automation (3). Although serologic typing is inferior to DNA typing in many aspects, it still has the advantage of detecting functional molecules in vivo. The non- or low-expressed alleles (4-7) as well as many novel alleles (8-11) have been identified by comparing the data obtained from serologic and DNA typing. In contrast, uninterpretable heterogeneous typing patterns of HLA molecule originating from the same allele have also been reported (12, 13). These imply that serologic heterogeneity can be attributed either to allelic differences or to other factors such as differences in the peptide bound to HLA molecules.

In Koreans, HLA-A24 is the second most frequently expressed HLA-A type (gene frequency, 22.8%) (14). Several different serologic reaction patterns have been observed in the routine typing laboratory. In this study, the source of A24 serologic heterogeneity identified in the Korean population was investigated using DNA sequencing analysis.

MATERIALS AND METHODS

Samples

Thirteen HLA-A24 positive DNA samples representing four different serologic reaction patterns were selected for the study. All individuals were unrelated except two siblings in a family exhibiting the same A24 variant pattern (A24v1) (Table 1). HLA-A,B,C types and HLA-DRB1 types of each sample were defined by serology using a commercial typing kit designed for Oriental populations (OT-72, One Lambda, Canoga Park, CA, U.S.A.) and a reverse PCR-SSOP typing system (15),

Table 1. Serologic reaction patterns and their partial amino acid sequences† of molecules encoded by selected A*24 alleles

Serologic pattern‡	Alloantisera			Allele§	A. A. residues‖				
	A9	A23	A24		62	65	127	166	167
A24 standard	8¶	1	8	A*2402101 (7)	E	G	K	D	G
A24 v1	8	1	1	A*2421†† (2)	-	-	N	-	-
A24 v2	1	1	8	A*2408 (3)	G	R	-	-	-
A24 v3	2	1	8	A*2403 (1)	-	-	-	E	W

†(17), ‡Reaction pattern was obtained using a commercial typing kit designed for Oriental populations (OT-72, One Lambda), §Defined by DNA sequencing analysis. The number of samples used in DNA sequencing analysis is indicated in the parentheses. ‖Only differences in exons 2 and 3 noted, ¶% of cytotoxicity detected in serologic typing, 8; 81-100%, 6; 51-80%, 4; 21-50%, 2; 11-20%, 1; <10%. ††Novel allele identified from two siblings in a family in this study. A*2421 differs from A*2402101 by a single nucleotide at codon 127 (AAA→AAC) resulting in an amino acid substitution (K→N).

respectively.

DNA sequencing analysis

Procedures and condition for DNA direct sequencing analysis using an automated DNA sequencer (Model 377, PE-Applied Biosystems Inc., Foster City, CA, U.S.A.) have been described (16). M13-tailed PCR primers were used to amplify the polymorphic regions of the HLA-A genes (exon 2, intron, exon 3) prior to performing DNA sequencing. The PCR product was used as a template for direct DNA sequencing using Cycle Sequencing Kits containing AmpliTaq DNA polymerase, FS (PE-Applied Biosystems Inc.). Cloning using the pCRII vector (Invitrogen, San Diego, CA, U.S.A.) was utilized to separate alleles in samples carrying a putative novel allele. Fluorescently labeled M13 forward and reverse primers as well as two internal sequencing primers derived from intron 2 (Int2f and Int2r) were used in the sequencing reaction to obtain the complete sequence information from both strands of exons 2 and 3. Sequences were analyzed using Sequence Navigator and MatchTool software (PE-Applied Biosystems Inc.).

RESULTS

Heterogeneity of the HLA-A24 serologic reaction patterns

The HLA-A, B, C serologic typing tray contained twenty HLA-A alloantisera including three different A9 antisera specific for A9, A23, and A24, respectively. As shown in Table 1, four different serologic reaction patterns were observed in the A24 positive Korean samples. The majority of the A24 samples showed the standard reaction pattern exhibiting strong positive reactivity with both A9 and A24 alloantisera. However, several samples showed variant reaction patterns by exhibiting strong positive reactivity to only one of the alloantiserum. Variants could be grouped into three groups (A24v1, A24v2, and A24v3) based on the serologic reaction patterns. While individuals in A24v2 and A24v3 groups were unrelated, the two individuals in a Group A24v1 were family members.

Serologic heterogeneity originated from different allelic products

DNA sequence analysis of the HLA-A24 genes clarified the serologic heterogeneity. As shown in Table 1, different A*24 alleles were identified in each serologic reaction group. A*2402101 were identified from seven samples exhibiting the standard A24 reaction pattern. The two sibling samples exhibited strong positive reactivity only to the A9 alloantiserum (A24v1), and appeared to carry a novel allele, A*2421. Samples exhibiting strong positive reactivity only to the A24 alloantiserum and negative (A24v2) or weak (A24v3) reactivity to the A9 alloantiserum carried either A*2408 or A*2403, respectively.

A24 variant allele, A*2421

Heterozygous sequencing histograms obtained from two siblings (DHL and JSL) exhibiting the A24v1 serologic reaction pattern identified a novel allele. A*2421 was most homologous to A*2402101 differing by a single nucleotide substitution at codon 127 (AAA→AAC) resulting in a predicted amino acid change from lysine (K) to asparagine (N) (Table 1). The novel allele, A*2421, was confirmed by sequence analysis of DNA obtained from seven and three isolated clones (pCRII vector, Invitrogen) derived from PCR amplification of individuals DHL and JSL, respectively. Based on the sequence, it is likely that the novel allele may have arisen either by a point mutation or by a gene conversion event involving A*2402101. A*2421 was submitted to the GenBank

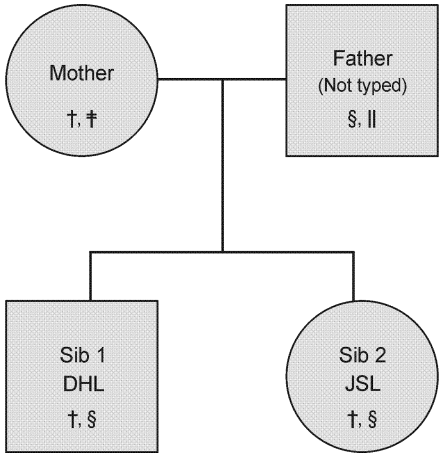

Fig. 1. Family tree and predicted HLA haplotypes in the family. HLA types of the three family members (mother, DHL, & JSL) were identified by serology (HLA-A,B,C only) and DNA sequencing analyses. Haplotype "||" could not be predicted because blood sample was not available from father (Haplotype: †A*03201(A32)-Cw*03031(Cw3)-B*1507(B62)-DRB1*0403-DRB4*01-DQB1*0302, ‡A*0207(A2)-Cw*0102(Cw1)-B*4601(B46)-DRB1*08032-DQB1*06011, §A*2421(A24v1)-Cw*03041(Cw3)-B*4002(B61)-DRB1*04051-DRB4*01-DQB1*0401, ||Not defined).

database and was assigned accession number AF106688 for exon 2 and AF106689 for exon 3. This allele was officially assigned by the WHO Nomenclature Committee in January 1999. The predicted haplotype associated with A*2421 seems to be A*2421-Cw*03041-B*4002-DRB1*04051-DRB4*01-DQB1*0401 from the family segregation study (Fig. 1).

Predicted antibody binding sites determining A9 and A24 serologic specificities

It was possible to localize the crucial binding sites for A9- and A24-specific antibodies by comparing the A24 serologic reaction patterns and putative amino acid sequences of A*24 alleles. As shown in Table 1, the molecule encoded by A*2402101 exhibited strong reactivity with both A9- and A24-specific alloantisera. However, the single substitution at residue 127 in A*2421 (K to N) abolished the binding of the A24-specific alloantiserum. This implies that residue 127K is an important site for the binding of A24-specific antibody. Likewise, substitutions at residues 62 (E to G) and 65 (G to R) in A*2408 abolished the binding of the A9-specific alloantiserum. Independently, substitutions at residues 166 (D to E) and 167 (G to W) in A*2403 reduced the binding activity to the A9-specific alloantiserum. This suggests that two physically separated sites, 62E-65G and 166D-167G, are important for the binding of A9-specific antibody.

DISCUSSION

This study demonstrated that the A24 serologic heterogeneity observed in the Korean population originated from four different A*24 alleles and each corresponded to different serologic reaction pattern. Among these, A*2402101 obtained from samples exhibiting the standard A24 serologic reaction pattern is expected to be the most frequent A*24 allele since majority of A24 positive Koreans exhibited the standard serologic reaction pattern. This prediction was confirmed by a population study using the PCR-SSOP typing method (data not shown). High frequencies of A*2402101 have also been reported in various human populations (18). This suggests that A*2402101 is most likely the original A*24 allele from which other A*24 alleles evolved. This hypothesis is supported by the observation that A*2402101 retains the conserved amino acids at polymorphic sites (localized in the α1 and α2 domains) that discriminate the A*24 alleles from one another (17). Comparing our data to those from Japanese, a closely related population (19, 20), two alleles (A*2402101 and A*2408) were identified in both populations. However, two alleles (A*2403 and A*2421) were additionally identified, while the other two alleles (A*2404 and A*2407) reported in Japanese were not identified in this study.

Comparison between serologic reaction patterns and amino acid sequences of specific HLA molecules has often been useful in predicting antibody epitopes (8, 10). This study also suggests three crucial sites for A9- and A24-specific antibody binding. First, 127K, located on an exposed loop of the α2 domain in the HLA-A molecule, appears to be a crucial site for the A24-specific antibody. However, it seems most likely that the antibody recognizes 127K together with the closely located 144K, 151H, and 156Q. The latter three residues differ from molecules carrying specificities other than A24 while 127K is also carried by other molecules including A2, A68, and A23. The possible role of 127K in antibody binding has also been postulated previously (21, 22). Secondly, 62E and 65G are crucial sites for A9-specific antibody binding. These sites have already been previously predicted as the A9 public epitope (23). Lastly, 166D and 167G also seem to be involved in the A9 epitope. Substitution at residues 62E-65G produces stronger effect on the serologic determinant than substitution at residues 166D-167G by abolishing rather than weakening the antibody binding reactivity (Table 1). This hypothesis should be tested by an expanded study using a larger number of samples carrying A*2403. 166D-167G has been postulated as the A23 and A24 related antibody binding site previously (23, 24). The 62E-65G and 166D-167G residues are located on a helices in the α1 and α2

domains, respectively, which are easily accessed by antibodies. These critical sites could directly affect antibody binding or alternatively, those residues could affect a conformational change resulting from either the amino acid substitution itself or by binding of different peptides involved in the antibody recognition site.

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