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Molecular Analyses of Five New Chimpanzee MHC Class I Alleles: Implications for Differences between Evolutional Mechanisms of HLA-A, -B, and -C Loci

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In order to study the origin of the polymorphism of MHC class I molecules, we have cloned and sequenced five new Patr-A, -B, and -C loci alleles from two chimpanzees. Previous studies of sequence comparison between Patr and HLA class I alleles revealed that many of the sequence motifs were shared and the origin of class I molecules predated the divergence of chimpanzees and humans. These findings are confirmed by our current study. Additionally, our data suggest significant differences between mechanisms of evolution of the A, B, and C loci: (1) The B locus is characterized by frequent nucleotide substitutions, whereas the A and C loci are relatively more conserved; (2) However, unlike the A locus, the α2 domains of the C locus sequenced appear to produce MHC polymorphism between these species. These differences might imply the distinctive contributions of each locus during the evolutionary history.

Major histocompatibility complex (MHC) class I molecules are cell-surface glycoproteins that play an essential role in the T cell-mediated immunity, including alloreactivity, anti-viral and anti-tumor responses. They also shape the T cell repertoire by positive and negative selections in the thymus. MHC class I molecules bind naturally processed antigenic peptides and present them to $CD8^+$ cytotoxic T lymphocytes (1). They are expressed as heterodimers composed of a variable heavy chain and an invariant light chain called β_2-microglobulin. The diversity of the heavy chain is thought to increase the number of potential antigenic peptides to be presented. However, the origin of the polymorphism is still obscure.

Comparison of alleles for class I genes in humans and other primates, including chimpanzees and gorillas, is a useful approach to study evolutionary mechanisms that generate the MHC polymorphism. Chimpanzees, *Pan troglodytes,* are the most closely related species to humans. It is well known that they possess the HLA-A, -B, and -C homologues, called Patr-A, -B, and -C, on the basis of sequence comparison (2). Certain HLA-A and HLA-B alleles are more closely related to particular Patr-A and Patr-B alleles than they are to other human A and B alleles, indicating that certain sequence motifs have been maintained over millions of years.

To explore further the origin of the polymorphism of MHC class I molecules, we have cloned and sequenced eight Patr-A, -B, and -C loci alleles including five previously undescribed alleles from two chimpanzees, and compared the five new nucleotide and amino acid sequences to those of various HLA alleles.

MATERIALS AND METHODS

Cell lines. Epstein Barr Virus (EBV)-transformed B cell lines, 1535-B and 1536-B, were established from the two chimpanzees, 1535 and 1536 (3) by standard methods. The B cell lines were maintained in RPMI 1640 medium supplemented with 10% FCS.

Isolation of mRNA. Total RNA was isolated from the B cell lines, 1535-B and 1536-B, using the RNA isolation reagent, ISOGEN (Nippon Gene, Japan). Briefly, 5×10^6 B cells were centrifuged and the pellet was resuspended in 1 ml of ISOGEN. The cells were then lysed by pipetting. Two-hundred microliters of chloroform was added and the mixture was vigorously vortexed. After 3 minutes' incubation at

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room temperature, the mixture was centrifuged and the aqueous phase was transferred into a new tube. RNA was precipitated by addition of 0.5 ml of isopropanol. The RNA pellet was washed with 70% ethanol, dried, and dissolved in 100 μl of DEPC-treated water.

Messenger RNA was isolated from the total RNA by use of Dynabeads Oligo $(dT)_{25}$ (Dynal, Oslo, Norway). One hundred microliters of the total RNA solution was heated to 65°C for 2 min to disrupt secondary structures. The RNA was mixed gently with 100 μl of prewashed Oligo $(dT)_{25}$ beads suspension. After washing the beads, the mRNA was eluted with 20 μl of 10 mM Tris, pH 7.5.

Preparation of first-strand cDNA. First-strand cDNA was synthesized by using the Oligo-(dT) primer and Moloney murine leukemia virus reverse transcriptase (M-MLV RT) (Promega, Madison, WI). In brief, 1 μl (0.5 μg) of the Oligo-$(dT)_{12-18}$ primer (Gibco BRL, Rockville, MD) and 12 μl of water was added to 2 μl of the mRNA solution. The mixture was heated at 70°C for 10 min and chilled down on ice for 5 min. Then, the following reagents were added: 2 μl (80 units) of RNasin (Promega, Madison, WI), 8 μl of 25 mM dNTP mix, 20 μl of 5× RT buffer (250 mM Tris-HCl, 15 mM $MgCl_2$, 375 mM KCl, 50 mM dithiothreitol), 10 μl of M-MLV RT (200 units/μl). After 90 min incubation at 42°C, the sample was heated to 95°C for 5 min to inactivate M-MLV RT.

Amplification of Patr class I cDNA by polymerase chain reaction (PCR). Patr class I cDNAs were amplified in a 50 μl mixture containing 2.5 μl of the first-strand cDNA, 5 μl of 10× dNTP mix (2 mM each), 1 μl of 50 μM 5′ and 3′ primers, 10× PCR buffer (Perkin-Elmer), $MgCl_2$ at a final concentration of 3 mM, 1.5 μl of Ampli-Taq Gold DNA polymerase (5 units/μl) (Perkin-Elmer). PCR primers were derived from the relatively conserved, untranslated regions that flank the 5′ and 3′ ends of the coding region of HLA-A, -B, and -C (sense: 5′-GGACTCACAATCTCCCCAGAC-3′; antisense: 5′-GAGGGAACACAGGTCAGTGTG-3′). Designing of these primers was based on the previous report (4) with a slight modification. These primers can be used for amplification of Patr-A, -B, and -C genes (manuscript in preparation). The amplification program used was: preheating [95°C, 9 min]; 35 cycles [95°C, 1 min; 55°C, 1 min; 72°C, 2 min]; last extension [72°C, 10 min].

Sequencing of Patr class I genes. The PCR products were cloned into the PCR II vector in the TA cloning kit (Invitrogen, San Diego, CA). The ligated PCR products were transfected into *E. coli* Top 10 by electroporation (Gene Pulser, BioRad, Richmond, CA), and positive plaques were selected on 2× YT plates containing ampicillin and X-gal. White colonies were picked from the plate and plasmid minipreparation was performed using QIAwell plasmid purification system (QIAGEN). Sequencing reactions were performed using the Thermo Sequenase fluorescent labeled primer cycle sequencing kit (Amersham) according to the manufacturer's instruction. Sequencing primers used were described previously (4, 5), and sequencing was performed in both directions. The PCR products were sequenced on the Shimadzu DSQ-1000L DNA sequencer (Shimadzu Ltd., Tokyo, Japan). To confirm there were no amplification errors in the sequences, we sequenced at least 5 clones per each allele.

RESULTS

We cloned and sequenced two Patr-A (35A-1, 36A-1), four Patr-B (35B-1, 35B-2, 36B-1, 36B-2), and two Patr-C (35C-1, 36C-1) genes from the two chimpanzees. Five (35A-1, 35B-1, 35B-2, 35C-1, and 35C-2) out of the eight alleles have previously been undescribed. The 36A-1, 36B-1, and 36B-2 correspond to the formerly characterized, Patr-A14, -B09, and -B01 alleles, respectively.

The HLA-A locus alleles are classified into six groups including HLA-A1/A3/A11, -A2, -A9, -A10, -A19, and -A80 families (6). Surprisingly, all Patr-A alleles thus far sequenced belong to only the A1/A3/A11 family (7–10), suggesting that chimpanzees may have inherited only part of the human HLA-A allelic repertoire. To explore this hypothesis, we have analyzed a new Patr-A locus allele, 35A-1. We have compared the nucleotide sequence of the Patr-A allele to those of various HLA-A locus alleles in the six HLA-A families (Table 1). As expected, the 35A-1 is more closely related to the A1/A3/A11 family than any other families. The 35A-1 is very similar to the HLA-A*1101 throughout the entire coding region. This allele differs from HLA-A*1101 by only 17 nucleotides (98.4% similarity) (Table 1), producing only 10 amino acid replacements (97.3% similarity) spread throughout the total sequence. The 35A-1 is also very similar to the other A1/A3/A11 family alleles, HLA-A*0101 and -A*0301 (23 and 19 nucleotide differences, respectively). In contrast, the 35A-1 differs from the other five HLA-A families by 33–52 nucleotides and by 24–34 amino acid residues. These results support the previous idea that the evolution of MHC polymorphism is a trans-species process (11) and the chimpanzee species may have been founded by a small population of animals with only a limited number of HLA-A1/A3/A11-related genes (10).

Four Patr-B alleles were cloned and sequenced from the chimpanzees, and two previously uncharacterized alleles, 35B-1 and 35B-2, have been identified. Consistent with the previous results (1, 8, 12), comparison of allelic sequences between the two novel Patr-B alleles and thirty-two HLA-B locus alleles demonstrates that there is no single HLA-B allele that is more similar to the Patr-B alleles over the entire length of the gene than other alleles (Table 2). This is not the case with Patr-A (Table 1; 7–10). These results suggest that, unlike the HLA-A locus, the HLA-B locus and its homologues are characterized by frequent nucleotide substitutions and/or recombinations. A characteristic of HLA-B molecules is the presence of the Bw4/Bw6-associated sequences at amino acid residues 74–83 of the α1 domain (13). The molecule encoded by 35B-1 has the sequence RIALR at positions 79–83 which forms the Bw4 specificity. On the other hand, the 35B-2 allele has the sequence GLRNLRG at positions 77–83 which differs from the typical Bw6-associated sequence (SLRNLRG) by only one amino acid residue.

In this study, two new Patr-C locus alleles, 35C-1 and 36C-1, have been identified from the chimpanzees. Surprisingly, comparison of the nucleotide sequences reveals that the α1 domain of 36C-1 differs from that of HLA-Cw*1701 by only two nucleotides, and the α3 and

TABLE 1

Nucleotide Differences in the 35A-1 Sequence

	LP	α1	α2	α3	TM	CP	Total
A1/3/11 family							
HLA-A*0101	3 (95.8)	7 (97.4)	8 (97.1)	2 (99.3)	2 (98.3)	1 (98.7)	23 (97.9)
HLA-A*0301	3 (95.8)	5 (98.1)	5 (98.2)	2 (99.3)	2 (98.3)	2 (97.4)	19 (98.3)
HLA-A*1101	3 (95.8)	6 (97.8)	3 (98.9)	2 (99.3)	2 (98.3)	1 (98.7)	17 (98.4)
HLA-A*3001	4 (94.4)	12 (95.6)	12 (95.7)	2 (99.3)	2 (98.3)	1 (98.7)	32 (97.1)
A2 family							
HLA-A*02011	5 (93.1)	9 (96.7)	14 (94.9)	13 (95.3)	3 (97.5)	1 (98.7)	45 (95.9)
HLA-A*68011	4 (94.4)	10 (96.3)	7 (97.5)	14 (94.9)	3 (97.5)	1 (98.7)	39 (96.4)
HLA-A*6901	4 (94.4)	10 (96.3)	13 (95.3)	13 (95.3)	3 (97.5)	1 (98.7)	44 (96.0)
A9 family							
HLA-A*2301	5 (93.1)	16 (94.1)	15 (94.6)	5 (98.2)	5 (95.8)	1 (98.7)	47 (95.7)
HLA-A*2402	5 (93.1)	16 (94.1)	11 (96.0)	5 (98.2)	4 (96.7)	1 (98.7)	42 (96.2)
A10 family							
HLA-A*2501	4 (94.4)	17 (93.7)	13 (95.3)	12 (95.7)	4 (96.7)	2 (97.4)	52 (95.2)
HLA-A*2601	4 (94.4)	10 (96.3)	13 (95.3)	12 (95.7)	4 (96.7)	2 (97.4)	45 (95.9)
A19 family							
HLA-A*2901	4 (94.4)	13 (95.2)	9 (96.7)	12 (95.7)	7 (94.2)	3 (96.2)	48 (95.6)
HLA-A*31012	4 (94.4)	9 (96.7)	9 (96.7)	11 (96.0)	7 (94.2)	2 (97.4)	42 (96.2)
HLA-A*3201	4 (94.4)	14 (94.8)	11 (96.0)	12 (95.7)	8 (93.3)	2 (97.4)	51 (95.3)
HLA-A*3301	4 (94.4)	10 (96.3)	9 (96.7)	12 (95.7)	7 (94.2)	2 (97.4)	44 (96.0)
A80 family							
HLA-A*8001	4 (94.4)	11 (95.9)	9 (96.7)	6 (97.8)	2 (98.3)	2 (97.4)	33 (97.0)

Note. Numbers of nucleotide substitutions between 35A-1/36A-1 and various HLA-A locus alleles. Numbers in parentheses are the percentage of nucleotide sequence identities in the given domains. LP: Leader Peptide; α1: α1 domain; α2: α2 domain; α3: α3 domain; TM: Transmembrane domain; CP: Cytoplasmic domain.

transmembrane domains of 36C-1 differ from those of HLA-Cw*0702 by only two and one (silent mutation) nucleotides, respectively (Table 3 and Fig. 1). The cytoplasmic domain of 36C-1 is identical to that of HLA-Cw*0702. These results suggest that intralocus recombinations might have occurred between the ancestral alleles of HLA-Cw*0702 and -Cw*1701. In contrast to these domains, the α2 domain of 36C-1 is not remarkably similar to the corresponding domain of any HLA-C locus allele (Table 3). Likewise, the α1 domain of 35C-1 is similar to that of HLA-Cw*1503 and HLA-Cw*1701 (3 and 4 nucleotide differences, respectively), whereas the α2 domain of 35C-1 is not similar to that of any HLA-C locus allele listed (Table 3). These data suggest that the α2 domain of 35C-1 and 36C-1 does undergo relatively frequent genetic exchanges and/or point mutations although the remaining domains are highly conserved. Interestingly, the nucleotide sequences of the α3 and cytoplasmic domains of an HLA-C homologue of Gorilla, Gogo-C*0202 are identical to those of 36C-1 (Table 3 and Fig. 1). Furthermore, the transmembrane domain of Gogo-C*0202 is very similar to that of 36C-1 (4 nucleotide and 2 amino acid differences). These data suggest that 36C-1, HLA-Cw*0702, and Gogo-C*0202 evolved from a common ancestral allele before separation of gorillas (7–10 million years ago).

DISCUSSION

Consistent with the previous results (7–10), the new Patr-A locus allele sequenced in this study belongs to the HLA-A1/A3/A11 family (Table 1). These results indicate that the ancestral alleles of the HLA-A1/A3/A11 family existed before the separation of humans and chimpanzees, and support the hypothesis that the evolution of the MHC has occurred slowly in a trans-species fashion (11). This suggests that either chimpanzees were founded by a limited number of individuals that expressed ancestral homologues of the HLA-A1, -A3, -A11 genes or that selective pressure of the environment has favored maintenance of chimpanzees with this particular family of alleles. Interestingly, gorilla HLA-A homologues are all related to the HLA-A2, A10, and A19 families and show no similarity to the A1/A3/A11, A9 families (14). In contrast to the A locus, the alleles of the HLA-B locus are relatively unstable. It has been proposed that the HLA-B locus undergoes rapid changes, especially in isolated human populations (15, 16). Analysis of the common and pygmy chimpanzee HLA-B homologues supports this notion (12). Our current results are consistent with these data. These results suggest that genetic changes frequently occurred in the B locus after their divergence from the ancestral gene, and therefore, the evo-

TABLE 2

Nucleotide Differences in the 35B-1 and 35B-2 Sequences

	35B-1			35B-2		
	α1	α2	Total	α1	α2	Total
HLA-B*07021	24 (91.1)	23 (91.7)	57 (94.8)	22 (91.9)	24 (91.3)	60 (94.5)
HLA-B*0801	26 (90.4)	19 (93.1)	53 (95.1)	19 (93.0)	20 (92.8)	51 (95.3)
HLA-B*1301	16 (94.1)	16 (94.2)	42 (96.1)	18 (93.3)	17 (93.8)	40 (96.3)
HLA-B*1302	16 (94.1)	12 (95.7)	38 (96.5)	18 (93.3)	13 (95.3)	36 (96.7)
HLA-B*1401	27 (90.0)	13 (95.3)	46 (95.8)	22 (91.9)	13 (95.3)	46 (95.8)
HLA-B*1402	26 (90.4)	13 (95.3)	45 (95.9)	21 (92.2)	13 (95.3)	45 (95.9)
HLA-B*15011	20 (92.6)	17 (93.8)	42 (96.1)	9 (96.7)	18 (93.5)	34 (96.9)
HLA-B*1801	26 (90.4)	12 (95.7)	47 (95.7)	18 (93.3)	14 (94.9)	35 (96.8)
HLA-B*2702	17 (93.7)	15 (94.6)	45 (95.9)	29 (89.3)	15 (94.6)	51 (95.3)
HLA-B*3501	21 (92.2)	15 (94.6)	46 (95.8)	12 (95.6)	17 (93.8)	35 (96.8)
HLA-B*3701	20 (92.6)	13 (95.3)	42 (96.1)	17 (93.7)	14 (94.9)	34 (96.9)
HLA-B*3801	22 (91.9)	12 (95.7)	40 (96.3)	27 (90.0)	13 (95.3)	51 (95.3)
HLA-B*39011	27 (90.0)	12 (95.7)	45 (95.9)	22 (91.9)	13 (95.3)	46 (95.8)
HLA-B*40011	23 (91.5)	23 (91.7)	57 (94.8)	16 (94.1)	24 (91.3)	54 (95.0)
HLA-B*4101	23 (91.5)	15 (94.6)	46 (95.8)	16 (94.1)	16 (94.2)	43 (96.1)
HLA-B*4201	24 (91.1)	19 (93.1)	51 (95.3)	22 (91.9)	20 (92.8)	55 (94.9)
HLA-B*4402	18 (93.3)	20 (92.8)	50 (95.4)	22 (91.9)	22 (92.0)	50 (95.4)
HLA-B*4501	23 (91.5)	16 (94.2)	43 (96.1)	16 (94.1)	17 (93.8)	41 (96.2)
HLA-B*4601	21 (92.2)	17 (93.8)	43 (96.1)	15 (94.4)	18 (93.5)	40 (96.3)
HLA-B*4701	20 (92.6)	15 (94.6)	49 (95.5)	18 (93.3)	14 (94.9)	40 (96.3)
HLA-B*4801	24 (91.1)	24 (91.3)	59 (94.6)	15 (94.4)	25 (90.9)	56 (94.9)
HLA-B*4901	17 (93.7)	13 (95.3)	34 (96.9)	22 (91.9)	13 (95.3)	43 (96.1)
HLA-B*5001	23 (91.5)	13 (95.3)	40 (96.3)	16 (94.1)	13 (95.3)	37 (96.6)
HLA-B*51011	17 (93.7)	13 (95.3)	40 (96.3)	19 (93.0)	13 (95.3)	38 (96.5)
HLA-B*52011	14 (94.8)	14 (94.9)	38 (96.5)	15 (94.4)	14 (94.9)	35 (96.8)
HLA-B*5301	15 (94.4)	15 (94.6)	40 (96.3)	18 (93.3)	17 (93.8)	41 (96.2)
HLA-B*5401	28 (89.6)	9 (96.7)	46 (95.8)	23 (91.5)	10 (96.4)	40 (96.3)
HLA-B*5502	25 (90.7)	9 (96.7)	43 (96.1)	20 (92.6)	10 (96.4)	37 (96.6)
HLA-B*5601	25 (90.7)	11 (96.0)	45 (95.9)	20 (92.6)	12 (95.7)	39 (96.4)
HLA-B*5701	8 (97.0)	15 (94.6)	34 (96.9)	23 (91.5)	17 (93.8)	47 (95.7)
HLA-B*5801	8 (97.0)	15 (94.6)	32 (97.1)	24 (91.1)	17 (93.8)	46 (95.8)
HLA-B*7801	21 (92.2)	13 (95.3)	43 (96.1)	14 (94.8)	10 (96.4)	32 (97.1)

Note. Numbers of nucleotide substitutions between 35A-1/36A-1 and various HLA-A locus alleles. Numbers in parentheses are the percentage of nucleotide sequence identities in the given domains. α1: α1 domain; α2: α2 domain. Total: the total sequence covering leater peptide, α1, α2, α3, transmembrane, and cytoplasmic domains.

lutional pathway of the HLA-B locus is distinct from that of the HLA-A locus.

The most prominent finding in the present study is that the C-terminal half (α3, transmembrane, and cytoplasmic domains) of 36C-1 is nearly identical to that of HLA-Cw*0702 as well as that of Gogo-C*0202 (Table 3 and Fig. 1). This strongly suggests that a common ancestral allele existed prior to divergence of gorillas (7–10 million years ago) which is followed by later divergence of chimpanzees and humans (5 million years ago). In addition, we have found that the α1 domain of 36C-1 is extremely similar to that of HLA-Cw*1701 instead of that of HLA-Cw*0702 (Table 3 and Fig. 1), which suggests that intralocus recombination might have occurred between the ancestral alleles of HLA-Cw*0702 and -Cw*1701. It seems that this recombination occurred just after divergence of chimpanzees and humans, since there is no single HLA-C locus allele that is closely related to 36C-1 over the entire domain. Alternatively, it is possible that HLA-Cw*0702, -Cw*1701 as well as Gogo-C*0202 diverged from an ancestral allele with 36C-1-like sequence that existed before separation of gorillas. In contrast to the stable α1, α3, transmembrane, and cytoplasmic domains, the α2 domain of 36C-1 is not remarkably similar to that of any HLA-C locus allele (Table 3 and Fig. 1). The same pattern is seen in the α2 domain of 35C-1 as well (Table 3). These data suggest that the ancestral α2 domains of 35C-1 and 36C-1 appear to have changed more frequently than other domains. Since the α2 domain forms a half part of a peptide-binding groove, it is likely that the rapid changes contributed to recognize newly encountered peptides. Taken together, it is likely that the HLA-C locus and its homologues were

TABLE 3

Nucleotide Differences in the 35C-1 and 36C-1 Sequences

	35C-1							36C-1						
	LS	α1	α2	α3	TM	CP	Total	LS	α1	α2	α3	TM	CP	Total
HLA-Cw*0102	1 (96.6)	13 (95.2)	11 (96.0)	2 (99.3)	2 (98.3)	1 (98.7)	30 (97.3)	2 (97.2)	11 (95.9)	12 (95.7)	10 (96.4)	8 (93.3)	2 (97.4)	45 (95.9)
HLA-Cw*02021	2 (97.2)	8 (97.0)	15 (94.6)	5 (98.2)	4 (96.7)	1 (98.7)	35 (96.8)	0 (100)	11 (95.9)	13 (95.3)	12 (95.7)	8 (93.3)	2 (97.4)	46 (95.8)
HLA-Cw*0302	2 (97.2)	6 (97.8)	15 (94.6)	3 (98.9)	2 (98.3)	1 (98.7)	30 (97.3)	1 (98.6)	8 (97.0)	12 (95.7)	10 (96.4)	7 (94.2)	2 (97.4)	40 (96.3)
HLA-Cw*03031	0 (100)	6 (97.8)	16 (94.2)	3 (98.9)	2 (98.3)	1 (98.7)	28 (97.4)	1 (98.6)	8 (97.0)	14 (94.9)	10 (96.4)	7 (94.2)	2 (97.4)	42 (96.2)
HLA-Cw*04011	0 (100)	8 (97.0)	17 (93.8)	3 (98.9)	4 (96.7)	1 (98.7)	33 (97.0)	1 (98.6)	8 (97.0)	13 (95.3)	10 (96.4)	10 (91.7)	2 (97.4)	44 (96.0)
HLA-Cw*0501	0 (100)	6 (97.8)	17 (93.8)	3 (98.9)	3 (97.5)	1 (98.7)	30 (97.3)	1 (98.6)	6 (97.8)	13 (95.3)	8 (97.1)	9 (92.5)	2 (97.4)	39 (96.4)
HLA-Cw*0602	0 (100)	7 (97.4)	13 (95.3)	3 (98.9)	4 (96.7)	1 (98.7)	28 (97.4)	1 (98.6)	5 (98.1)	13 (95.3)	8 (97.1)	8 (93.3)	2 (97.4)	37 (96.6)
HLA-Cw*0701	3 (95.8)	9 (96.7)	10 (96.4)	11 (96.0)	11 (90.8)	3 (96.2)	47 (95.7)	2 (97.2)	7 (97.4)	9 (96.7)	2 (99.3)	3 (97.5)	0 (100)	23 (97.9)
HLA-Cw*0702	3 (95.8)	8 (97.0)	11 (96.0)	11 (96.0)	11 (90.8)	3 (96.2)	47 (95.7)	2 (97.2)	6 (97.8)	10 (96.4)	2 (99.3)	1 (99.1)	0 (100)	21 (98.2)
HLA-Cw*0801	0 (100)	10 (96.3)	15 (94.6)	3 (98.9)	2 (98.3)	1 (98.7)	32 (97.1)	1 (98.6)	10 (96.3)	11 (96.0)	8 (97.1)	9 (92.5)	2 (97.4)	41 (96.2)
HLA-Cw*12021	0 (100)	6 (97.8)	15 (94.6)	2 (99.3)	4 (96.7)	1 (98.7)	28 (97.4)	1 (98.6)	6 (97.8)	12 (95.7)	9 (96.7)	8 (93.3)	2 (97.4)	38 (96.5)
HLA-Cw*1301	0 (100)	6 (97.8)	16 (94.2)	3 (98.9)	4 (96.7)	1 (98.7)	30 (97.3)	1 (98.6)	8 (97.0)	12 (95.7)	8 (97.1)	8 (93.3)	2 (97.4)	39 (96.4)
HLA-Cw*14021	0 (100)	11 (95.9)	15 (94.6)	2 (99.3)	3 (97.5)	1 (98.7)	31 (97.2)	1 (98.6)	10 (96.3)	12 (95.7)	10 (96.4)	8 (93.3)	2 (97.4)	43 (96.1)
HLA-Cw*1503	1 (96.6)	3 (98.9)	16 (94.2)	2 (99.3)	4 (96.7)	1 (98.7)	27 (97.5)	0 (100)	7 (97.4)	12 (95.7)	9 (96.7)	8 (93.3)	2 (97.4)	38 (96.5)
HLA-Cw*1601	0 (100)	7 (97.4)	11 (96.0)	3 (98.9)	8 (93.3)	1 (98.7)	30 (97.3)	1 (98.6)	7 (97.4)	9 (96.7)	11 (96.0)	10 (91.7)	2 (97.4)	40 (96.3)
HLA-Cw*1701	4 (94.4)	4 (98.5)	17 (93.8)	7 (97.5)				3 (95.8)	2 (99.3)	13 (95.3)	11 (96.0)			
HLA-Cw*1801	3 (95.8)	7 (97.4)	17 (93.8)	2 (99.3)	4 (96.7)	1 (98.7)	34 (96.9)	2 (97.2)	5 (98.1)	13 (95.3)	9 (96.7)	8 (93.3)	2 (97.4)	39 (96.4)
Gogo-C*0202	0 (100)	6 (97.8)	9 (96.7)	9 (96.7)	12 (90.0)	3 (96.2)	39 (96.4)	1 (98.6)	6 (97.8)	10 (96.4)	0 (100)	4 (96.7)	0 (100)	21 (98.2)

Note. Numbers of nucleotide substitutions between 35C-1/36C-1 and various HLA-C locus alleles. Numbers in parentheses are the percentage of nucleotide sequence identities in the given domains. LP: Leader Peptide; α1: α1 domain; α2: α2 domain; α3: α3 domain; TM: Transmembrane domain; CP: Cytoplasmic domain.

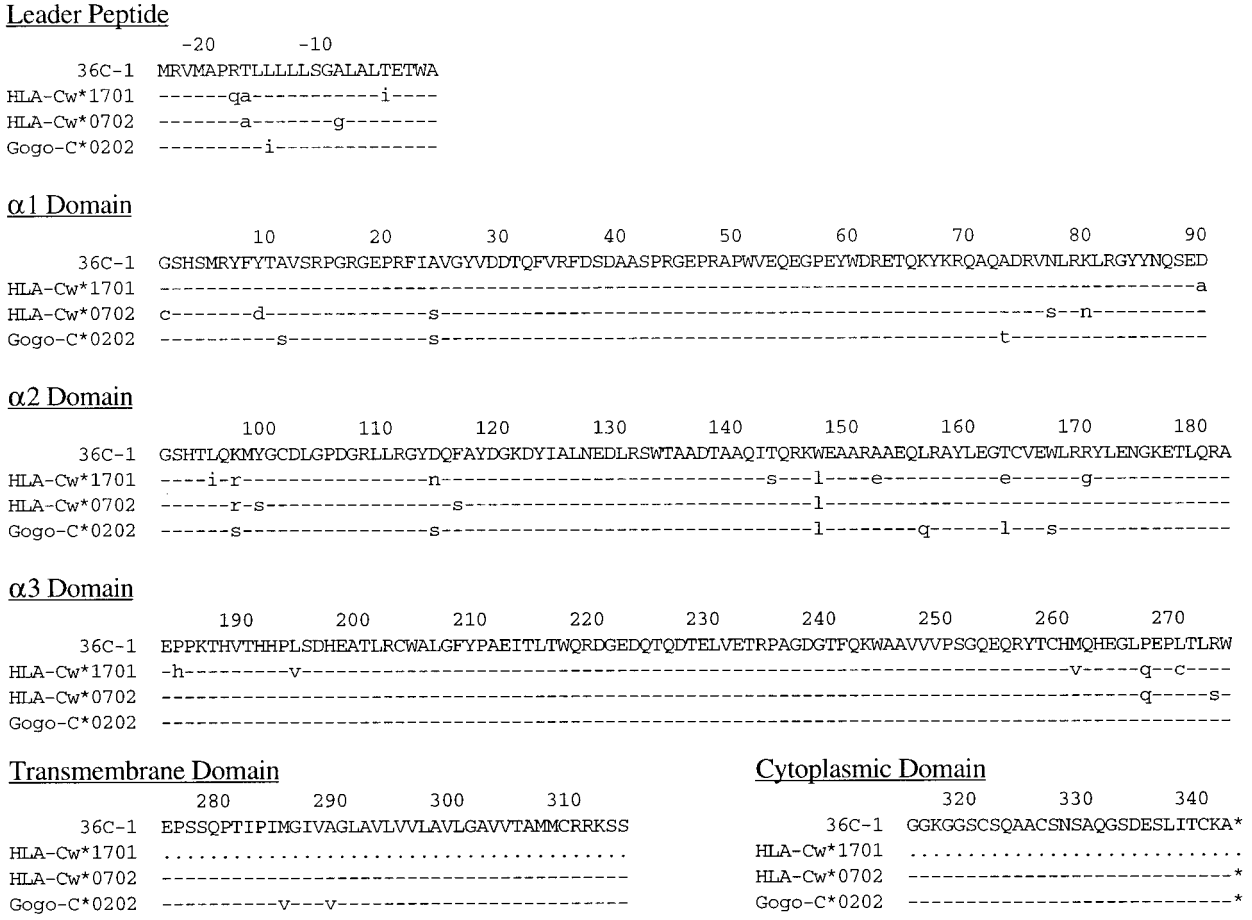

FIG. 1. Amino acid sequence comparison of 36C-1 with HLA-Cw*1701, HLA-Cw*0702, and Gogo-C*0202. Hyphens indicate identity with the sequence of 36C-1. Missing sequence is indicated by a period.

very stable in the evolutionary history. However, unlike the HLA-A locus, it seems that the entire sequence of the C locus is not conserved. In addition, Patr-A locus alleles are not related to any gorilla HLA-A homologues at all (14), whereas the C-terminal half of 36C-1 is very similar to Gogo-C*0202 (Table 3). Taken together, these suggest that the evolutionary process of HLA-C is different from that of HLA-A. Recently, it was reported that an MHC class I C locus allele was shared by the pygmy chimpanzee and the common chimpanzee (17), indicating remarkable conservation of C locus alleles.

Comparison of the allelic polymorphism and sequence variability in human and chimpanzee class I genes shows that many of the sequence motifs are shared and the origin of class I molecules predate the divergence of these species. However, our current data and the previous data (12, 15, 16) suggest significant differences between mechanisms of evolution of the A, B, and C loci, implying the distinctive contributions of each locus during the evolutionary history.

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