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A VERTICALLY TRANSMITTED HIV-1 GAG-SUBTYPE VARIANT DETECTED IN TAIWAN

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SUMMARY: We used polymerase chain reaction (PCR) to amplify a segment, about 560 base pairs (bp), of HIV-1 gag DNA prepared from peripheral blood mononuclear cells of a seropositive Taiwanese pair of mother and infant. TM-1 and TC-1 clones of PCR-amplified DNA derived from the mother and infant, respectively, showed a 94.5% homology with each other. However, the TM-1 and TC-1 sequences exhibited lower degrees of homology, i.e. only 85.1% and 85.8%, respectively, with the corresponding gag segment of a North American HIV-1 subtype (HXB2), and 86.4% and 87.0%, respectively, with that of a Zairean HIV-1 subtype (Z2Z6). The divergence of TM-1 and TC-1 sequences from those of HXB2 and Z2Z6 is particularly prominent in the first (5' proximal) 200 bp of the cloned DNA segment, involving transitions more frequently than transversions. Two additional clones TM-2 and TC-2 derived from the mother and infant were sequenced for the first 200 bp. These four clones showed a high degree of homology (94.7-97.5%) among themselves, providing an evidence for transmission of the virus from the mother to the infant. These findings show the epidemiological value of PCR, and indicate the presence of a gag subtype of HIV-1 which is distinct from both the North American and Zairean subtypes according to the phylogenetic tree constructed.

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INTRODUCTION

Genetic variants of human immunodeficiency virus type 1 (HIV-1) arise during viral replication by error-prone reverse transcription (1,2). From an infected individual, different isolates may exhibit high degrees of variability (3,4). The complex mixture of quasispecies (5,6) in an infected individual may lead to selection of a variant on the basis of high replication efficiency with different degrees of cytopathic effects (7-9), specific cellular tropism (10-13) or capability to escape from the immunologic defense mechanisms (14-16). Most of these variabilities are reflected as changes in *env* gene sequences. In contrast, the *gag* sequence is more conserved among different isolates of HIV-1 (17).

Perinatal transmission of HIV-1 is estimated to occur in 13-39% of infants born to infected mothers (18-20). The polymerase chain reaction (PCR) for detection of HIV-1 genome in these infants would be a more accurate diagnostic measure of infection than the HIV-1 antibody tests, since the maternal antibody in the peripheral blood of an uninfected infant may give a false positive test. We have cloned the *gag* sequences amplified by PCR from the DNA of the peripheral blood mononuclear cells obtained from a Taiwanese mother and her infant daughter who were both found to be seropositive for HIV-1 infection (21). The cloned *gag* sequences from them were found to be closely related to each other, and thus strongly suggest that the infant was infected through vertical transmission from her mother. While *gag* DNA sequences obtained from some other seropositive Taiwanese individuals were closely related to those of North American HIV-1 subtype (unpublished data), those derived from the mother-child pair were, unexpectedly, quite distinct from known North American and Zairean subtypes. Their phylogenetic relationships are presented in this report.

MATERIALS AND METHODS

Blood specimens: A heparinized peripheral blood sample was collected in April, 1989 from a 25-year old Taiwanese woman with a persistent lymphadenopathy, and from her infant daughter (6-month old), who were both found to be seropositive (confirmed by Western blot) for HIV-1 in December, 1988. The woman had lived with a man of German nationality who is now suspected to be a bisexual. He has left her since, and is not available for test. The high

molecular weight DNA was extracted from the mononuclear cells which were separated from erythrocytes by Ficoll-Hypaque method.

PCR-amplification, cloning and sequencing: DNA samples (2 μ g) were subjected to repeated rounds of amplification (30 cycles) in a 100- μ l reaction mixture containing 100 pmol each of SK37 primer (26-mer sense strand: ATACAGTAGCAACCCTCTATTGTGTG) and SK39 primer (28-mer antisense strand: TTTGGTCCTTGTCTTATGTCCAGAATGC) (22), [nucleotides 1027 to 1052 and 1657 to 1630, respectively, of the HXB2 sequence (17)], 200 μ M each deoxynucleotide, 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM MgCl₂, and 2 U of Taq DNA polymerase. Each cycle consisted of a 2-min denaturation step at 94 C, followed by a 2-min annealing step at 50 C, and a 2-min extension at 72 C, except that the first cycle used a 5-min denaturation at 94 C, and the last cycle used a 7-min extension at 72 C. Aliquots (5 μ l) of the PCR mixture were mixed with 5 μ l of distilled water and 1 μ l of stop buffer, and electrophoresed on 0.8% agarose gels containing 0.5 μ g of ethidium bromide per ml. After electrophoresis, gels were denatured, neutralized, and transferred to nylon membrane filters, which were then prehybridized by standard method and hybridized with ³²P-labeled SK19 probe (22) (41-mer sense strand: ATCCTGGGATTAAATAAAATAGTAAGAATGTATAGCCCTAC) (nucleotides 1587 to 1627 of the HXB2 sequence). The filters were autoradiographed and if hybridization results were positive, the amplified DNA was cloned. The multiple cloning site of pBluescript II SK +/- was cut with *EcoRV* and dephosphorylated before ligation with the PCR-amplified fragments which were treated with polynucleotide kinase. The recombinant plasmid was used for transformation of *E. coli* HB101 and screened by colony-hybridization with SK19 probe. The positive colony was harvested and minipreparations were prepared for DNA sequencing by standard dideoxy method. All sequences were resolved on buffer gradient gels, and both plus- and negative-strands were sequenced.

RESULTS AND DISCUSSION

Two clones of the PCR-amplified *gag* fragment of DNA derived from the Taiwanese woman (TM-1 and TM-2) and two from her infant daughter (TC-1 and TC-2) were analyzed. As shown in Fig. 1, the sequences for TM-1 (563 bp) and TC-1 (562 bp) were compared with the corresponding sequence (nucleotides 1066 to 1629) of the North American subtype, HXB2 (17,23), and that (nucleotides 1067 to 1633) of the Zairean subtype, Z2Z6 (17). It is evident that while TM-1 and TC-1 sequences were quite similar with each other, they showed quite a number of dissimilarities when compared with those of HXB2 and Z2Z6. These

HXB2	1066	AGATAAAGA	CACCAAGGAA	GCTTTAGACA	AGATAGAGGA	AGAGCAAAAC	AAAAGTAAGX
Z2Z6	1067	--G-----	-----	-----A-	----G-----	---A-----	-----A
TM-1	1	-TG-----	-----A--	---C---AG	-AG---AA-	--CA-----G	--C--C---
TC-1	1	-TG-----	-----A--	---C---GG	-AG-G--AA-	--CA---GG	--C--C---
HXB2	1125	XXXXXXXXAGC	ACAGCAAGCA	GCAGCTGACA	CAGGACACAG	CAATCAGGTC	AGCCAAAATT
Z2Z6	1127	AC-----G--	-----	-----G	----GA---A	--GC-----	-----
TM-1	59	--C-----A-	-----G---	---AG---GG	A-A--A---	--GC--A---	-T-----
TC-1	59	--C-----A-	-----G---	---AG---G-	A---A---	--GC--A---	-T-----
HXB2	1183	ACCCATATAGT	GCAGAACATC	CAGGGGCAAA	TGGTACATCA	GGCCATATCA	CCTAGAACTT
Z2Z6	1187	-T-----	-----C-A	-----	-----	-----	-----
TM-1	118	-----	-----TGA	--A-----X	CC-----C-	-----	-----
TC-1	118	-----	-----XX--	--A-----C	C-A---C-	-----	-----
HXB2	1243	TAAATGCATG	GGTAAAGTA	GTAGAAGAGA	AGGCTTTCAG	CCCAGAAGTG	ATACCCATGT
Z2Z6	1247	-G--C-----	-----	A-----A-	-----	-----A	-----
TM-1	177	-G---A-----	-----	-----G-A-	---C-----	T-----A	-----
TC-1	176	-G-----	-----	-----A-	---C-----	T-----A	-----
HXB2	1303	TTTCAGCATT	ATCAGAAGGA	GCCACCCAC	AAGATTTAAA	CACCATGCTA	AACACAGTGG
Z2Z6	1307	-----	-----	-----	-----	T-----	-----
TM-1	237	-----	-----G	-----	-----	---T-----	-----A-
TC-1	236	-----	-----G	-----	-----	--T-TA-TC-	-----
HXB2	1363	GGGGACATCA	AGCAGCCATG	CAAATGTTAA	AAGAGACCAT	CAATGAGGAA	GTCGCAGAAT
Z2Z6	1367	-----	-----	-----C---	-G-----	-----	-----
TM-1	299	-AA-G-----	-----T--A	-----C---	-G---T--A-	-----A--	-----G-
TC-1	298	---G-----	-----T--	-----C---	-G---T--A-	-----	-----G-
HXB2	1423	GGGATAGAGT	GCATCCAGTG	CATGCAGGGC	CTATTGCACC	AGGCCAGATG	AGAGAACCAA
Z2Z6	1427	-----GT-	A-----	-----	-----	-----C-	-----
TM-1	357	A---C--GA-	A-----CCA	--A-----	-----	---T-----A	---T---
TC-1	356	---C--GA-	A-----CCA	--A-----	-----	---T-----A	---T---
HXB2	1483	GGGGAAGTGA	CATAGCAGGA	ACTACTAGTA	CCCTTCAGGA	ACAAATAGGA	TGGATGACAA
Z2Z6	1487	-----	T-----	-----	-----	-----C-	-----
TM-1	417	-A-----	T-----	-----	---A-----	-----A--	-A-----C-
TC-1	416	-----	T-----	-----	---A-----	-----A--	-----C-
HXB2	1543	ATAATCCACC	TATCCCAGTA	GGAGAAATTT	ATAAAAGATG	GATAATCCTG	GGATTAAATA
Z2Z6	1547	GC--C-----	-----	-----C-	-----	-----	-----
TM-1	477	GC-----	-----	-A-----	-----A	-----A	A-----
TC-1	476	GC-----	-----	-----	-----	-----	-----
HXB2	1603	AAATAGTAAG	AATGTATAGC	CCTACCA			
Z2Z6	1607	-----	-----	---GT--			
TM-1	537	-----	-----	---GT--			
TC-1	536	-----	-----	---GTT-			

Fig. 1. Nucleotide sequence alignments for HXB2, Z2Z6, TM-1 and TC-1 *gag* sequences. -: identical to HXB2. X: a gap inserted to maintain alignment.

dissimilarities were quite prominent in the segment representing the first (5' proximal) 200 bp. Therefore, two more clones, TM-2 and TC-2, derived also from the mother and her infant were sequenced for 199 and 200 bp, respectively, and compared with the corresponding segment of TM-1 and TC-1 sequences, as illustrated in Fig. 2. These sequences were quite similar with each other, giving 94.7-97.5% homologies as compared among themselves. Therefore, these clones may represent a set of quasispecies present in each individual (5,6). As reported by Goodenow et al. (5), these changes in nucleotides involved transitions more frequently than transversions. The close relationship between these four clones as shown by the high degree of homologies among themselves suggests strongly that the infant was infected with the vertically transmitted HIV-1 from her mother. Since the infant was delivered by cesarian section and bottle-fed (21), it is highly probable, but not proven, that intrauterine or intrapartum infection occurred. Since only a few clones were examined, it is difficult to say whether a selective transmission of a particular quasispecies from the mother to the infant occurred, as reported recently by Wolinsky et al (24). Nevertheless, it is evident that PCR-amplification and sequencing is valuable for epidemiological tracing of sources of HIV-1 infection. These *gag* sequences isolated from the mother-infant pair may represent defective viruses because of the deletions and mutations observed. The presence of such defective clones of quasispecies has also been described by others (5) although the significance of such defective viruses in the pathogenesis of AIDS is not clear.

The interrelationships in terms of homologies among TM-1, TC-1, HXB2 and Z2Z6 *gag* sequences are presented in Fig. 3. While TM-1 sequence showed a 94.7% homology to that of TC-1, it exhibited lower similarities to those of HXB2 and Z2Z6, giving homologies of 85.1% and 86.4%, respectively. TC-1 sequence also showed rather low homologies, i.e. 85.8% and 87.0%, respectively, to those of HXB2 and Z2Z6. The HXB2 sequences showed a 93.7% homology to that of Z2Z6, the former representing a North American subtype and the latter a Zairean subtype. As shown in Fig. 4, the phylogenetic tree which was constructed on the basis of our TC-1 sequence compared with a spectrum of other sequences (17), the TC-1 sequence stands out as the most distant cousin of BRU sequence which is quite similar to that of HXB2. In fact, the TC-1 sequence is not only distinct from the viruses BRU, HXB2, JH31, RF, OYI and JRCSF which would cluster as North American subtype, but also quite different from those of ELI, Z2Z6, NDK, and MAL viruses which would cluster as Zairean subtype. It is of interest to point out that the TC-1 sequence is positioned in the phylogenetic tree between the

TM-1	1	ATGTAAAAGA	CACCAAAGAA	GCTCTAGAAX	GAAGTAGAAA	AAGCACAAAA	GAACAGTCAG
TM-2	1	-----	-----	-----G-	-----	-----G	-----
TC-1	1	-----	-----	-----G-	-----G-	-----G	-----
TC-2	1	-----	-----	-----GG	-----G-	-----	-----
TM-1	60	CAAAAAACAC	AGCAGGCAGC	AAGTGAGGAA	AGAAACAGCA	GCCAAGTCAG	TCAAAATTAC
TM-2	60	--C-----	-----T	-----	G-----	-----	-----
TC-1	60	-----	-----	-----A-	G-----	-----	-----
TC-2	61	-----	-----	-----	G-----	-T-----	-----
TM-1	120	CCTATAGTGC	AGAACTGACA	AGGGCAACCG	XTACACCAGG	CCATATCACC	TAGAACTTTG
TM-2	120	-----	---X----	-----	-----	-----	-----
TC-1	120	-----	---X-CX--	-----	A-----	-----	-----
TC-2	121	-----	---X-CG--	-----	TC-----	-----	-----
TM-1	179	AATACATGGG	TAAAAGTAGT	AG			
TM-2	178	-----	-----	--			
TC-1	178	---G-----	-----	--			
TC-2	180	-X-G-----	-----	--			

Fig. 2. Nucleotide sequence alignments for TM-1, TM-2, TC-1 and TC-2 *gag* sequences. -: identical to TM-1. X: a gap inserted to maintain alignment.

Fig. 3. Interrelationships in terms of degrees of similarity among the *gag* sequences of TM-1, TC-1, HXB2 and Z2Z6.

Fig. 4. Phylogenetic tree of HIV-1 *gag* sequences including TC-1 sequences. Sequences of BRU, HXB2, JH31, RF, OYI and JRCSF cluster as North American subtype, while ELI, Z2Z6, NDK, and MAL sequences are included in the Zairean subtype. CPZ, which is a simian immunodeficiency virus (SIV) isolated from a chimpanzee, is related to HIV-1. [See Ref. (17) for origins of these viruses.]

Zairean subtype and the CPZ virus, which is an HIV-1-like simian immunodeficiency virus (SIV) isolated from a chimpanzee (17,25). Recently, it was proposed by G. Myers that, based on our TM-1 and TC-1 sequence supplied to the database, these (Taiwanese strains) would represent one of the five “families” of viruses (26). The other four “families” would include (i) US and European strains, (ii) Brazilian and Zairean strains, (iii) Zambian and Somalian strains, and (iv) the strains from Uganda, Ivory Coast and Kenya (26).

It may be speculated that the Taiwanese woman was infected from the man of German nationality who could have been the carrier of a distinct subtype of

HIV-1. Unfortunately, this person and the Taiwanese mother-infant pair are not available for further study, and the amount of the original sample of DNA was not sufficient for further analysis of sequences other than *gag*. However, we are using portions of the known TM-1 and TC-1 sequences as primers and probes to determine the prevalence of this subtype of HIV-1 among various populations.

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