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Nucleotide Sequence Variation of Human T-Lymphotropic Virus Type II in Vietnam

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A high rate of human T-lymphotropic virus type II (HTLV-II) infection has been documented in intravenous drug abusers (IVDAs) in South Vietnam. We have investigated the molecular characteristics of the virus and have shown that one HTLV-II subtype is predominant in Ho Chi Minh City. This molecular subtype, HTLV-IIb, was identified in a number of South Vietnamese by nucleotide sequence analysis of the long terminal repeat (LTR) region. HTLV-IIa was not found. These findings suggest that HTLV-IIb is endemic in IVDAs in South Vietnam, although IVDAs in urban areas in North America are predominantly infected with HTLV-IIa.

Key words: LTR sequence variation of HTLV-II — HTLV-IIb — IVDAs in South Vietnam

A high rate of human T-lymphotropic virus type II (HTLV-II) infection occurs in intravenous drug abusers (IVDAs) in Vietnam, Europe and urban areas of North America.[1–5] Further, HTLV-II infection is endemic in the Indian population of North America,[6,7] as well as South America.[8,9] Molecular analyses of the viruses in these groups have indicated that there are at least 3 subtypes, HTLV-IIa, -IIb, and -IIc, which can be differentiated by nucleotide sequence analysis.[10] HTLV-IIc was reported to be more related to HTLV-IIa than HTLV-IIb in terms of the sequences of the long terminal repeat (LTR) and *env* region, though the Tax protein of HTLV-IIc is more similar to that of HTLV-IIb because they both have an additional 25 amino acids at the carboxyl terminus.

In previous studies on serological analysis of HTLV-II infection in Southeast Asia, we demonstrated that IVDAs of South Vietnam, but not North Vietnam or Central Thailand, were infected with HTLV-II. We suggested that the HTLV-II in South Vietnamese IVDAs appeared to be a mixture of subtypes a and b, with subtype a being predominant, based on the use of a subtype-specific peptide enzyme-linked immunosorbent assay (ELISA).[3] In this study we have attempted to characterize the HTLV-II in the IVDAs of South Vietnam by nucleotide sequence analysis of the 5′ LTR in the virus.

Blood samples were collected from 20 blood donors, of whom 6 were IVDAs and 14 were non IVDAs, in Ho Chi Minh City in South Vietnam in 1996 (Table I). Plasma and peripheral blood mononuclear cells (PBMC) were separated by using Ficoll-paque.[11] All 6 serum specimens of our IDVA participants were positive in the gelatin particle-agglutination test (Serodia-ATLA, Fujirebio Inc., Tokyo) for HTLV in a primary screening assay followed by type specific HTLV immunoblotting (HTLV BLOT 2.3, Diagnostic Biotechnology Pte Ltd., Singapore).[1–3] Two of the 6 sera positive for HTLV demonstrated antibodies to various HTLV antigens and were positive for antibodies to HTLV-II rgp46-II and rgp21 but negative for antibodies to HTLV-I rgp46, suggesting that these 2 IVDAs were seropositive for HTLV-II, but did not have double infection with HTLV-I and HTLV-II. However, the other 4 of the 6 specimens were serologically indeterminate, showing positive with only one of the two kinds of recombinant proteins of HTLV-II. All 14 sera from non IVDAs from South Vietnam were seronegative for HTLV antigens (data not shown).

Genomic DNA of PBMC was extracted and purified with InstaGene Purification Matrix (Bio-Rad, Hercules, CA) for polymerase chain reaction (PCR) analysis. The positions of the oligonucleotides are numbered relative to the MoT isolate[12] in the Entrez data base, National Center for Biotechnology Information, National Library of Medicine (National Institute of Health, Bethesda, MD). The DNA of HTLV-II 5′ LTR region was amplified by nested PCR by using the following outer primer pairs; OA (nt 194–218; 5′-CCTTACCCACTTCCCCTAGCACTGA-3′) and OD (nt 807–831; 5′-GGGAAA-

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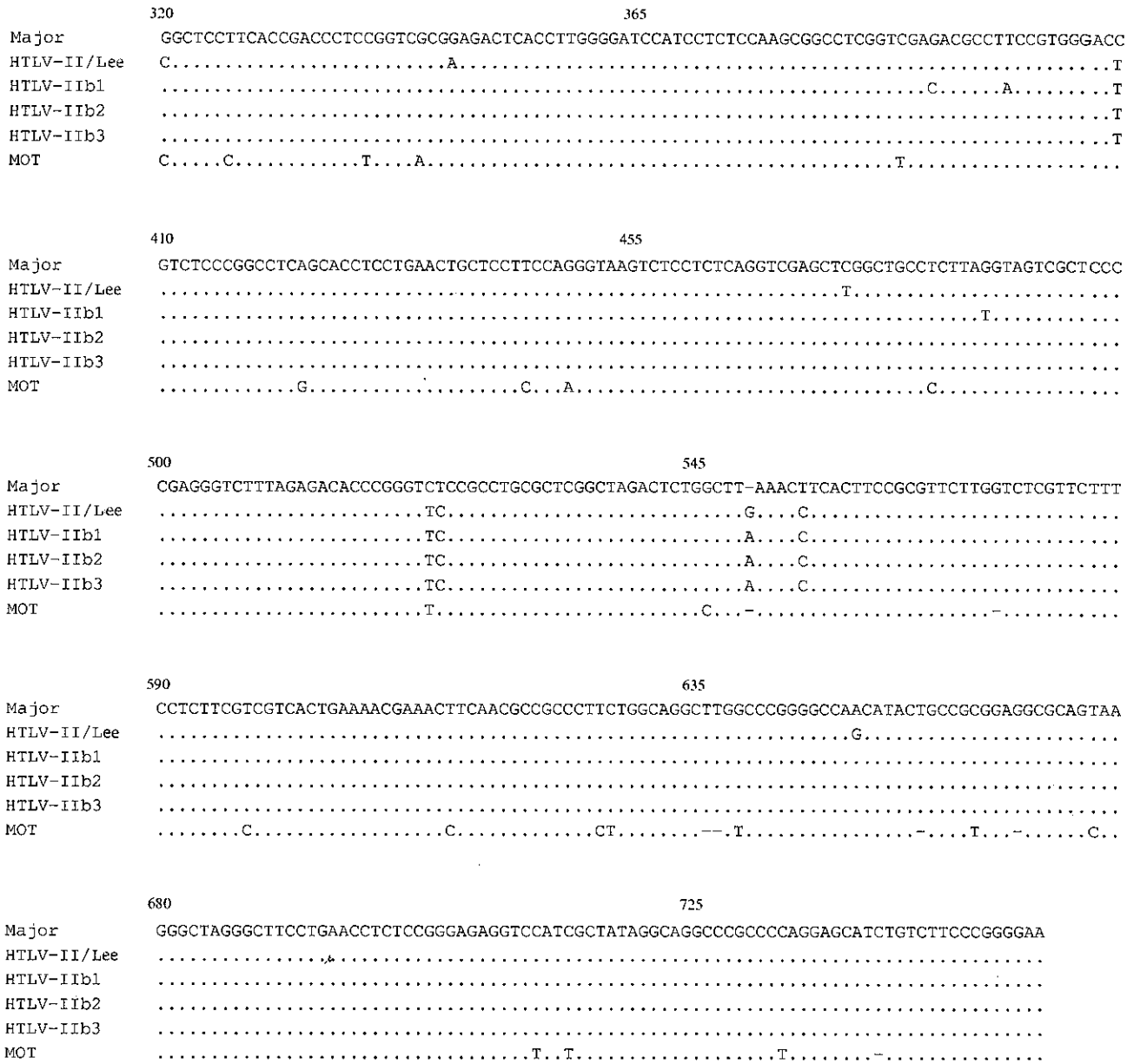

Fig. 1. Alignment of nucleotide sequences of HTLV-II from IVDAs in South Vietnam. The 5′ LTR sequences of the genomic DNA from 3 IVDAs with HTLV-II are aligned with that of HTLV-II Major,[13] HTLV-II subtype IIb HTLV-II/Lee[16] and HTLV-II subtype IIa isolate MoT.[12] The positions of the oligonucleotides are numbered relative to the MoT isolate in the ENTREZ database, National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD. Dots represent identity with the Major sequence, and the dashes represent nucleotide insertions or deletions.

GCCCGTGGATTTGCCCCAT-3′).[13] IB (nt 290–314; 5′-AAAAGCGC AAGGACAGTTCAGGAGG-3′) and IC2 (nt 759–783; 5′-ATCCCGGACGAGCCCCCACG-GGTTT-3′) were used as inner sets of primer pairs in the nested PCR.[13] β-Actin PCR was carried out as a PCR control.[14,15] The thermal profile of the nested PCR was 96°C for 1 min, 55°C for 2 min, and 72°C for 1 min at 35 times. As summarized in Table I, 3 of the 6 IVDAs (both of those seropositive for HTLV-II and one of those serologically indeterminate for HTLV-II) were identified

Table I. Record and Serological Findings of Blood Donors Positive for HTLV-II

Subject no.	Age (Sex)	Nationality	Risk factor	Anti-HTLV antibody PA		HTLV-II LTR PCR	Anti-HTLV antibody WB (IgG)						
				HTLV-I	HTLV-I/II		rgp21	p19	p24	p28	p53	HTLV-I rgp46	HTLV-II rgp46
1	34 (M)	Vietnamese	IDU	128	++	+	+w	+w	++	−	−	−	++
2	40 (M)	Vietnamese	IDU	64	+w	−	−	+w	−	−	−	−	−
3	41 (M)	Vietnamese	IDU	128	+	+	−	+	+	+	+	−	−
4	36 (M)	Vietnamese	IDU	256	++	−	+w	+w	+w	−	−	−	−
5	32 (M)	Vietnamese	IDU	64	++	−	−	−	−	−	−	−	++
6	43 (M)	Vietnamese	IDU	128	++	+	+	++	++	++	++	−	++

PA, gelatin particle-agglutination test; WB, western immunoblot.
The intensity of protein band in the western blot test was graded as follows: −, negative; +w, weakly positive; +, moderately positive; ++, strongly positive.

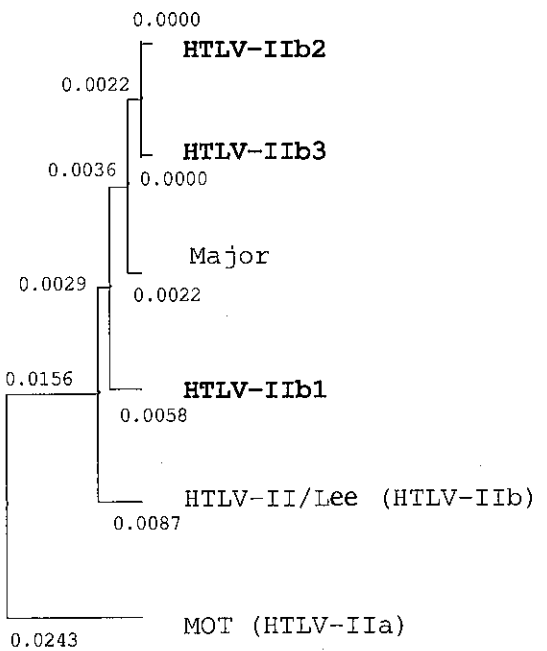

Fig. 2. Phylogenetic relationship of the 5′ LTR regions of the HTLV-II genes from IVDAs in South Vietnam. The phylogenetic pattern was obtained by the UPGMA method using PHYLIP based on approximately 450 nucleotides of the LTR region of the HTLV-II gene. The numbers indicate the separation of the clones.

as positive by Southern blot analysis after PCR of HTLV-II LTR. Genomic DNA from MoT cells cultured for 4 days, kindly supplied by Dr. T. M. Folks, Retrovirus Diseases Branch, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA, USA, was used as a reference specimen of HTLV-II, and that from H9/HTLV-IIIMN (AIDS Research and Reference Reagent Program, NIH, Rockville, MD) was used as negative specimen (data not shown).

The PCR results were molecularly confirmed by DNA sequencing. PCR products were purified by preparative agarose gel electrophoresis and sequencing was performed by the dye-terminator method with a 373AS automated DNA sequencing system (Applied Biosystems, Foster City, CA). Nucleotide sequences were edited and translated by using Factura and Sequence Navigator Software (Applied Biosystems, Inc.).[14,15] The nucleotide sequences of HTLV-IIb1, HTLV-IIb2, HTLV-IIb3 and MoT were obtained, and compared with the consensus of HTLV-II Major[13] as well as HTLV-II/Lee[16] (Fig. 1). The nucleotide sequences of the three IDVAs, HTLV-IIb1, -IIb2 and -IIb3, were all within the reported range of sequence diversity of HTLV-IIb.[13] Further, we detected two kinds of HTLV-IIb in South Vietnam by nucleotide sequencing of the HTLV-II 5′ LTR region, because the sequences of HTLV-IIb2 and HTLV-IIb3 were identical, while that of HLTV-IIb1 was different (Fig. 1). Nucleotide deletions and insertions were not detected in this region in the 3 HTLV-IIb-positive individuals.

Phylogenetic tree analysis was performed by the UPGMA method using PHYLIP.[6] Within the HTLV-IIb subtype, HTLV-IIb1, HTLV-IIb2 and HTLV-IIb3 were clearly demonstrated to cluster with HTLV-II/Lee. HTLV-IIb1 was in a different cluster from HTLV-IIb2 or HTLV-IIb3 (Fig. 2), suggesting that at least 2 kinds of HTLV-IIb are present in IVDAs in South Vietnam. HTLV-IIa subtype MoT was phylogenetically distinct (Fig. 2). Accession numbers for the sequences of HTLV-IIb1, -IIb2, and -IIb3 are AB001979, AB001980 and AB002532, respectively.

These results indicate the presence of IVDAs with HTLV-IIb but not HTLV-IIa in South Vietnam, based on both serological and molecular analysis. Previous

studies from our laboratory demonstrated that approximately 60% of IVDAs were seropositive for HTLV-II, and both HTLV-IIa and HTLV-IIb subtypes were identified in this population, with a predominance of HTLV-IIa, by employing HTLV-IIb-specific peptide-based ELISA.[3] The present results suggest that the data obtained from the ELISA assay may have limited usefulness in evaluating HTLV-II subtypes.

The IVDAs in North America are infected with predominantly HTLV-IIa,[6,7] and HTLV-IIb predominates in the Paleo-Indians in North America and Indian populations in Central and South America.[8,9] HTLV-IIb seropositive Japanese individuals have also been identified in Japan.[17,18] However, in other East or Southeast Asian countries, such as Mongolia[19] and Indonesia,[20] HTLV-IIa has been found exclusively by serological and/or genetic methods. The present results, together with our previous serological analyses[1,2] of HTLV-II subtypes suggest that HTLV-IIb is endemic in IVDAs in South Vietnam.

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