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Subtype Analysis of HTLV-1 in Patients with HTLV-1 Uveitis

Ayako Ono,[1,2] Tomoyuki Miura,[3] Shinji Araki,[4] Kazunari Yamaguchi,[5] Kiyoshi Takatsuki,[5] Sigeo Mori,[1] Masanori Hayami,[3] Manabu Mochizuki[2] and Toshiki Watanabe[1,6]

[1]Department of Pathology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, [2]Department of Ophthalmology, Kurume University, School of Medicine, 67 Asahimachi, Kurume, Fukuoka 830, [3]Institute for Virus Research, Kyoto University, 53 Shogoin-Kawaramachi, Sakyo-ku, Kyoto 606, [4]Miyata Eye Hospital, 6-3 Kuraharamachi, Miyakonojo, Miyazaki 885 and [5]Department of Internal Medicine, Kumamoto University, School of Medicine, 1-1-1 Honjo, Kumamoto, Kumamoto 860

The hypothesis that HTLV-1 uveitis, a recently identified disease entity associated with human T-cell leukemia virus type I (HTLV-1), is caused by a specific subtype of the virus was tested. The nucleotide sequences of the long terminal repeat of HTLV-1 from five patients with HTLV-1 uveitis (HU) and four with adult T-cell leukemia were phylogenetically analyzed. Our results showed that both subtypes which had been identified in Japan were associated with HU, indicating that there was no difference in pathogenicity between these phylogenetic subtypes. One of the subtypes was more frequently isolated in Okinawa than in Kyushu, suggesting a bias in the prevalence of each subtype among the inhabitants of these two areas of Japan.

Key words: HTLV-1 uveitis — ATL — LTR sequence — phylogenic subtype

Human T-cell leukemia virus type I (HTLV-1) is a human retrovirus that is causatively associated with a unique T cell malignancy, adult T-cell leukemia (ATL)[1-5] and the chronic progressive neurologic disorder, tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM).[6,7] In addition to these, we have recently reported a new disease entity caused by HTLV-1, HTLV-1 uveitis (HU), based upon seroepidemiological, ophthalmological and virological studies.[8-12] HU was defined as an idiopathic uveitis of otherwise asymptomatic HTLV-1 carriers and is characterized by the sudden onset of mild iritis, moderate or severe vitreous opacities, and mild retinal vasculitis in one or both eyes.

Thus, HTLV-1 is associated with at least three distinct disease entities: ATL, TSP/HAM and HU. The question of whether the same virus could cause both malignant transformation of the infected cells resulting in ATL and chronic inflammatory disorders such as TSP/HAM and HU, remains to be answered. As for TSP/HAM, neither a specific variant of HTLV-1 nor any specific mutation in the nucleotide sequence has been identified.[13-16] However, the notion of a relationship between nucleotide sequence variations of retroviruses and their pathogenicity remains valid. It is known that the cell tropism of murine leukemia virus is altered by minor nucleotide changes in the long terminal repeat (LTR), and that the virus changes from a leukemic to a neuropathic form when the nucleotide sequence in the *env* region is altered.[17,18]

Based upon the phylogenetic analyses of the LTR sequences, we grouped the viruses of the HTLV family into several subtypes, of which two have been found in Japan.[19] Other groups have reported a few HTLV-1 subtypes, and they also found two of them in Japan.[13,20] These observations, as well as the presence of the new disease HU, which is distinct from ATL and TSP/HAM, led us to test the specificity of the pathogenicity of each subtype in terms of ATL and uveitis. Our results indicated that there is no disease specificity among the phylogenetic subtypes, but that there is a bias in the distribution of HTLV-1 subtypes in Japan.

We determined the nucleotide sequences of the LTR region of nine HTLV-1 provirus clones; five were from HU, and four were from ATL patients. Six patients were born in Kyushu and 3 in Okinawa, where HTLV-1 is highly endemic (Table I). The LTR sequences were amplified by polymerase chain reaction (PCR)[21] with two sets of primer pairs. The nucleotide sequences of the primers are as follows: L1, 5′-TGACAATGACCATGAGCCCCA-3′ (nucleotide position 1–21), L2, 5′-TGTGGTGCCTCCTGAACTGCG-3′ (nucleotide position 439–459), L3, 5′-CGATCTGTAACGGCGCAGAAC-3′ (complementary sequence of nucleotide position 621–601), L4, 5′-GTGTACTAAATTTCTCTCCTG-3′ (complementary sequence of nucleotide position 754–734). After 50 reaction cycles, the amplified sequences were separated by electrophoresis on a 2% NuSieve (FMC Bioproducts, ME) agarose gel in TBE buffer. They were extracted from the gel with Geneclean II (BIO 101 Inc.,

[6] To whom correspondence should be addressed.

CA) and subcloned into the pCRII vector of the TA cloning kit (In Vitrogen, CA). The nucleotide sequences of the insert DNA were determined using a Cycle Sequence kit (Applied Biosystems, CA) and an automatic sequencer (370A DNA Sequencer, Applied Biosystems). Sequences from more than two plasmid clones for each PCR product were analyzed to discriminate between *in vivo* mutations and base substitutions due to misincorporation by *Taq* polymerase. A comparison of the sequences with that of the original HTLV-1 isolate, λATK,[22] is shown in Table II. There were four positions, 128, 209, 210 and 316, at which the nucleotide was uniformly substituted in almost all the sequences. The position 733/734 seemed to be deleted in the λATK clone, because all the LTR sequences that we studied contained an extra C at this position. Other than these positions, mutations seemed to be distributed randomly throughout the LTR region, showing no apparent clustering. They are generally located outside the transcription control elements such as the 21 bp repeat,[23] the Ets-1[24] and Myb binding sites.[25] However, some base

Table I. Patients from whom PBMC Samples Were Collected

No.	Patient	Sex	Age	Diagnosis	Birthplace
1	MA	F	67	ATL[a]	Kyushu
2	NA	M	49	ATL	Okinawa
3	KU	M	40	ATL	Kyushu
4	CI	F	32	ATL	Okinawa
5	HI	F	44	HU[b]	Kyushu
6	HA	F	50	HU	Kyushu
7	IW	M	24	HU	Kyushu
8	SA	F	41	HU	Kyushu
9	KR	F	40	HU	Okinawa

a) ATL: adult T cell leukemia.
b) HU: HTLV-1 uveitis.

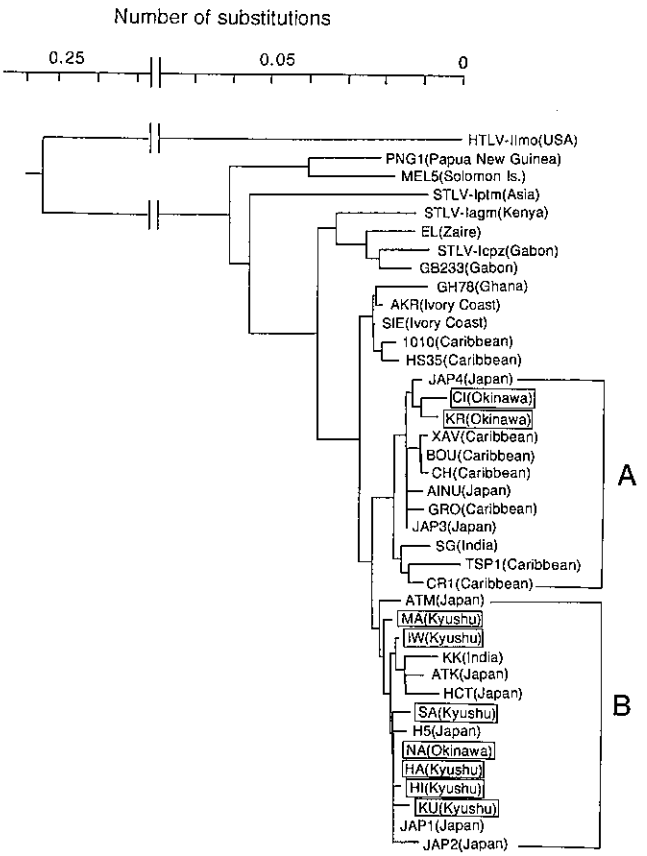

Fig. 1. A phylogenetic tree of the HTLV-1 family based upon LTR sequence variations. This tree was constructed by neighbor joining[27] after aligning the nucleotide sequence of part of the LTR region (144–650 in λATK[22]) using the ODEN software at the National Institute of Genetics in Japan. Nine isolates in this study (boxed) were classified into A and B subtypes. The scale on the tree is the number of nucleotide substitutions estimated by the six-parameter method,[29] and the horizontal branch length indicates the genetic distance.

Table II. Base Substitutions in the LTR

Patient	Nucleotide position																														
	67	116	128	141	146	150	151	209	210	211	239	246	278	297	306	316	317	320	350	438	481	498	562	581	609	612	667	721	725	733/734
ATK	A	T	G	G	G	T	A	A	A	G	C	T	A	C	C	G	C	G	A	C	A	G	A	A	C	T	G	C	T	X
1			A		A			G	G							A				T										C
2		C	A					G	G							A														C
3			A			C		G	G							A						A							C	C
4	C		A	A	A		G	G	G	X		C		G	G	A		A	C		G			G	T	C		T		C
5			A					G	G		A					A														C
6			A					G	G							A														C
7			A						G							A											A			C
8		C	A					G								A							G						C	C
9	C		A	A	A			G	G			C	G		G	A	G				G			G	T	C		T		C

X: positions where nucleotide is deleted.

substitutions were found in these elements. Nucleotide position 67 is located in the upstream Ets-responsive region (ERR-2)[24] and was substituted by C in clones 4 and 9. The nucleotide position 209–211 is located in the middle of the tax-responsive element (TRE)-2S,[26] and both of the As at 209 and 210 were substituted by G in 8 of the 9 clones studied. However, TRE-2S was characterized using sequences having G at both of these positions. The functional significance of the base substitutions described above, if any, is not clear at present.

Clones 4 and 9 had more base substitutions (15 and 12, respectively) than the other clones, which had 1 to 3 mutations, except for the 5 nucleotide positions noted above. These two clones shared the same base substitutions at 10 positions, suggesting a closer relationship with each other than with the rest of the clones. Base substitutions in clones 4 and 9 made them resistant to restriction enzyme digestion by *Mae*II, *Mae*III and *Dra*I, which is compatible with "subtype II" reported by Komurian-Pradel *et al.*[20] The overall sequence homology of the LTR sequence (nucleotides 22 to 733) of these two clones to that of λATK was 97.2 and 97.6% in clones 4 and 9, respectively, whereas those of the other clones were between 99.3% and 98.9%. These results suggested that these two clones are distinctive and belong to another subtype of HTLV-1. This notion was confirmed by a computer analysis of the relationship of these LTR sequences to those reported (Fig. 1), showing that the seven more conserved clones belonged to the B subtype and the two more divergent clones to the A subtype. This phylogenetic tree was constructed by the neighbor joining method[27] based upon the 6-parameter method of estimating nucleotide substitution.[28] No substantial change in the phylogenetic tree was found when it was constructed by unweighted pair grouping (UPG).[29] These results demonstrated that HU is associated with both HTLV-1 subtypes, indicating that there is no difference in their pathogenicity. Thus, the different pathogenicity cannot be ascribed to evolutionarily distinguished subtypes of HTLV-1, although this does not exclude the possibility of disease-specific mutation(s) in the HTLV-1 gene.

Our study also suggested a bias in the distribution of these subtypes in Japan, because two of the three clones from Okinawa, Nos. 4 and 9, belonged to HTLV-1 subtype A, which consists of Indian, Japanese and Caribbean isolates, whereas all six clones from Kyushu belonged to subtype B, which consists of Japanese isolates, except for one Indian isolate (Fig. 1). We identified three isolates of the A subtype in Japan, one Ainu in Hokkaido, and two TSP/HAM patients in Kagoshima, Kyushu, the most southerly of the four main islands of Japan (Fig. 1 and reference 19). The other 6 Japanese clones characterized in our previous study belonged to subtype B. This study added the sequence information from 9 isolates, and in a total of 18 Japanese isolates, 5 were of the A, and 13 of the B subtype. Therefore it is conceivable that the more prevalent subtype in Japan is B, with A being a minor subtype that is prevalent mainly in the most northerly and southerly regions. This is of interest from the anthropological perspective that the inhabitants of these areas, the Ainu and Ryukyuans, are considered to be the descendants of native Japanese populations.[30] Our results are compatible with the hypothesis that the A subtype of HTLV-1 was prevalent among the native Japanese who were later expelled to the north or south by the newer inhabitants, the Wajin, who constitute most of the current Japanese population.

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