Absence of HTLV-II Co-infection in HTLV-I-associated Myelopathy Patients

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Syncytium inhibition assay to distinguish between HTLV-I and HTLV-II infection was performed with sera and cerebrospinal fluid of 15 HTLV-I-associated myelopathy (HAM) patients. Also, genome analysis of HTLV-II proviral DNA was performed in some HAM patients by use of the polymerase chain reaction method. All of the HAM patients were negative for co-infection with HTLV-II.

Key words: Syncytium inhibition assay — HAM patient — HTLV-II — Polymerase chain reaction

Human T-cell leukemia virus type I (HTLV-I) and human T-cell leukemia type II (HTLV-II) have been associated with adult T-cell leukemia (ATL), HTLV-I-associated myelopathy (HAM) or tropical spastic paraparesis and a T-cell variant of hairy-cell leukemia.[1–4] HTLV-I has been found to be highly endemic among patients and healthy carriers in certain regions of Japan. On the other hand, only two patients have been found to have HTLV-II infection in unusual T cell malignancies resembling hairy-cell leukemia.[4, 5] Recently, high rates of HTLV-II infection were found in seropositive intravenous (IV) drug users (DUs) in New Orleans and Italy, in Guaymi Indians in Panama and in HAM patients.[6–10] The anti-HTLV-I antibody screening methods that have been used are the gelatin particle agglutination (PA) test, the indirect immunofluorescence assay, the enzyme-linked immunosorbent assay (ELISA) and the western blot test. On the other hand, although it is difficult to distinguish between HTLV-I and HTLV-II serologically because of their relatively high amino acid sequence homology, the application of polymerase chain reaction (PCR) techniques can differentiate the viruses by identifying type-specific proviral sequences. However, PCR technology is not yet suitable for the analysis of large numbers of blood samples. We have previously observed that HTLV-II-infected T-cells exhibit syncytium formation upon co-cultivation with HTLV-II-uninfected T-cells.[11] Also, the syncytium formation was inhibited by sera of HTLV-II-positive carriers but not inhibited by sera of ATL patients. These results should provide a basis to distinguish between HTLV-I and HTLV-II infection. The syncytium inhibition assay was first described by Clapham et al.[12]

Here, we present a syncytium formation inhibition test to distinguish between HTLV-I and HTLV-II infection in HAM patients. Also, genome analysis of HTLV-II was performed in some of the HAM patients by use of the PCR method. The patients with HAM were 13 women (mean age 61, range 45–78) and 2 men (aged 41 and 65). Titration values of anti-HTLV-I antibody in the sera and cerebrospinal fluid (CSF) were measured by PA test. The titration values in the sera and CSF were in the ranges of ×64 to >×2048 and ×16 to >×2048, respectively (Table I). Molt-4 T-cells (8×10^4 cells) were cocultivated with 4×10^4 HTLV-II-infected T-cell line (HTLV-IIC) cells in each well of a 96-well plate and sera or CSF were added to each sample at the final concentration of 1:20 or 1:10 dilution at the time of seeding. Co-cultivation was done for 24 h.

None of the sera or CSF samples in HAM patients inhibited syncytium formation (Fig. 1 and Table I). On the other hand, serum samples from HTLV-II-seropositive IV DUs used as positive controls inhibited syncytium formation.

Leukocytes from 10 ml of peripheral blood from 12 of the 15 HAM patients were separated on a Ficoll-Conray gradient. To detect the HTLV-II provirus genome in the primary cells, DNA from these cells was analyzed by a previously described PCR method combined with the digoxigenin (Dig)-ELISA method.[13] Briefly, DNA was prepared from samples of approximately 10^6 cells of each case by sodium dodecyl sulfate-proteinase K treatment, followed by phenol and chloroform extraction. Then one microgram of DNA was subjected to 30 cycles of PCR amplification. A primer pair complementary to a conserved region of pol (SK58/SK59) was used in the amplification of the HTLV-II DNA sequence.[14, 15] The designated primer SK58 is complementary to the viral

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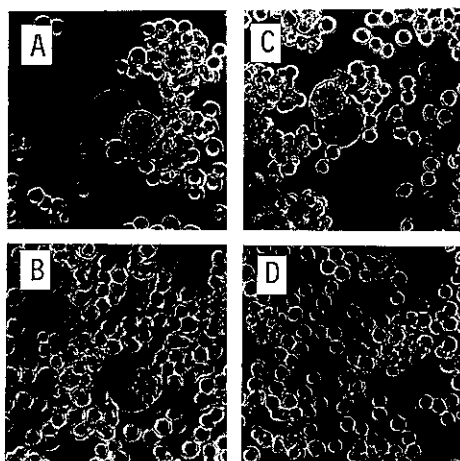[4] To whom correspondence should be addressed.

Fig. 1. A, B, C and D show the appearance of syncytium formation produced by co-cultivation of Molt-4 T-cells and HTLV-IIC cells, the absence of inhibition by anti-HTLV-I antibody-positive serum from an ATL patient, the absence of inhibition by anti-HTLV-I antibody-positive serum from a HAM patients, and the inhibition of syncytium formation by anti-HTLV-II antibody-positive serum from an IV DU positive control, respectively.
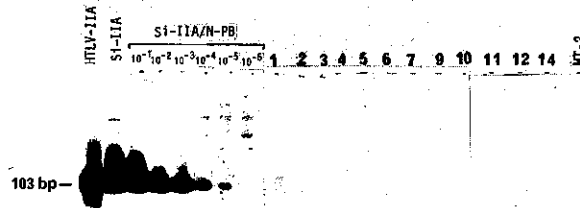

Fig. 2. Detection of the HTLV-II provirus genome in HAM patients. The sensitivity of the PCR method in detecting HTLV-II genome in HTLV-II-infected cells was demonstrated. DNAs from HTLV-II-infected Si-IIA cell line and leukocytes in peripheral blood from a normal person were prepared by phenol and chloroform extraction. One microgram of Si-IIA DNA was serially diluted at 1/10 dilution with DNA from a normal person (N-PB). Then $1 \mu g$ of DNA was subjected to 30 cycles of PCR amplification. Lanes 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12 and 14 were cases no. 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12 and 14. The primers SK58 and SK59 and probe SK60 were used in the PCR reaction.

Table I.   Results of HTLV-II Syncytium Inhibition Assay and PCR Genome Analysis in HAM Patients

Case no.	Source	Age/sex	HTLV-I PA method		HTLV-II syncytium inhibition test		HTLV-II PCR genome analysis
			Sera	CSF	Sera	CSF	
1	HAM	51/F	$> \times 1024$	$\times 16$	$< \times 20$	$< \times 10$	—
2	HAM	58/F	$\times 1024$	$\times 4 \, (-)$	$< \times 20$	ND	—
3	HAM	78/F	$> \times 1024$	$> \times 1024$	$< \times 20$	$< \times 10$	—
4	HAM	65/F	$\times 1024$	$\times 4 \, (-)$	$< \times 20$	ND	—
5	HAM	45/F	$> \times 1024$	$\times 256$	$< \times 20$	$< \times 10$	—
6	HAM	69/F	$> \times 2048$	$> \times 2048$	$< \times 20$	$< \times 10$	—
7	HAM	60/F	$\times 64$	$\times 2 \, (-)$	$< \times 20$	ND	—
8	HAM	68/F	$> \times 2048$	$> \times 2048$	$< \times 20$	$< \times 10$	ND
9	HAM	60/F	$> \times 2048$	$> \times 2048$	$< \times 20$	$< \times 10$	—
10	HAM	60/F	$> \times 2048$	$\times 16$	$< \times 20$	$< \times 10$	—
11	HAM	66/F	$\times 512$	$\times 64$	$< \times 20$	$< \times 10$	—
12	HAM	54/F	$> \times 2048$	$\times 16$	$< \times 20$	$< \times 10$	—
13	HAM	53/F	$> \times 2048$	$\times 256$	$< \times 20$	$< \times 10$	ND
14	HAM	41/M	$> \times 2048$	$\times 1024$	$< \times 20$	$< \times 10$	—
15	HAM	65/M	$> \times 2048$	$> \times 2048$	$< \times 20$	$< \times 10$	ND
16	IV DU	unknown	$> \times 512$	ND	$\times 20$	ND	+
17	IV DU	unknown	$> \times 512$	ND	$> \times 2560$	ND	+
18	IV DU	unknown	$\times 512$	ND	$\times 320$	ND	+
19	IV DU	unknown	$> \times 512$	ND	$\times 160$	ND	+
20	ATL patient	54/F	$\times 128$	ND	$< \times 10$	ND	ND
21	HTLV-I carrier	42/M	$\times 128$	ND	$< \times 10$	ND	ND
22	HTLV-I carrier	55/M	$\times 128$	ND	$< \times 10$	ND	ND

CSF; cerebrospinal fluid. ND; not done. −; negative.

minus strand and SK59 to the plus strand. After amplification, 10 μl of the reaction mixture was used for further analysis by 1.8% agarose gel electrophoresis, followed by denaturing in alkaline solution and transfer onto a "Hybond" (Amersham) nylon membrane. After pre-hybridization and hybridization with the 3'-end Dig-11-dUTP-labeled SK60 probe, the filter was washed, blocked with blocking reagent, reacted with polyclonal sheep anti-Dig fragments conjugated to alkaline phosphatase and colored with nitro blue tetrazolium and 5-brom-4-chloro-3-indolyl phosphate solution. The SK60 probe was a *pol* region oligonucleotide in the HTLV-II provirus DNA sequence. The PCR primers used in this experiment are similar to those used by one group,[9] but different from those used by another group.[10]

A 103-base-pair portion of HTLV-II-specific amplified DNA product was not detected in the examined HAM samples (Fig. 2). However, it was detected in positive controls (HTLV-IIA and Si-IIA cell lines).[13, 15]

Recently, two groups in Japan reported high rates of co-infection with HTLV-I and HTLV-II in patients with HAM by use of PCR technology.[9, 10] However, Kiyokawa *et al.* found no co-infection with HTLV-I and -II in HAM patients and ATL patients by use of an ELISA method.[16] Our results also showed the absence of co-infection with HTLV-I and -II in HAM patients by use of syncytium formation inhibition assay and PCR technology. Further studies are needed to resolve the conflict.

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