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Defective human T-cell lymphotropic virus type I (HTLV-I) provirus in seronegative tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) patients

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Abstract

Infection with human T-cell lymphotropic virus type I (HTLV-I) have been associated with the development of the tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM). We studied the presence of HTLV-I provirus in peripheral blood mononuclear cells (PBMC) from 72 Chilean patients with progressive spastic paraparesis by polymerase chain reaction: 32 seropositive and 40 seronegative cases. We amplified different genomic regions of HTLV-I using primers of 5′ *ltr*, *tax*, *env/tax*, *pX*, *pol* and *env* genes. These genes were detected from all seropositive patients. The seronegative patients were negative with 5′ *ltr*, *pol*, *env*, and *pX* primers. However, amplified product of tax and env/tax genes was detected from 16 and four seronegative patients, respectively. Three of them were positive with both genetic regions. The results of this study show that the complete HTLV-I provirus is found in 100% of seropositive cases. In seronegative cases, clinically very similar of seropositive cases, was found only tax gene in 42.5% (17/40) of patients. These results suggest the presence of a defective HTLV-I provirus in some seronegative patients with progressive spastic paraparesis, and suggest a pathogenic role of this truncate provirus for a group of TSP/HAM.

Keywords: Defective provirus; HTLV-I; TSP/HAM patients

1. Introduction

Tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) is a neurological disease defined by a progressive slowly spastic paraparesis that begins normally at fifth decade of life and it predominates in women. A generalized hyperreflexia Babynski signs, spastic hypertonus, clinically characterize it; the weakness involves the lower limbs and their shows uncommonly sensitive symptoms (Gessain et al., 1985; Cartier et al., 1992). However, the 75–80% of patients have altered the somato-sensory evoked potentials (SSEP), showing a subclinic disorder in the sensitivity (Castillo et al., 1999). Cerebrospinal fluid (CSF) is normal in 65% of TSP/HAM, only the 35% of patients have an increase of lymphocytes in CSF (Nakagawa et al., 1995). Neuroradiological studies show some atrophy of the spinal cord without signs of desmielinate disease (Ferraz et al., 1997). Neuropathological studies show degeneration of cortico-spinal tract (axomielinic degeneration) in all of them, and also compromise the cervical segments of the posterior columns in some patients. Only 50% of the case have the presence of lymphocytes in the perivascular areas in the spinal cord (Cartier et al., 1997).

We have detected in Chile one of the highest prevalence of TSP/HAM cases over the world (Cartier and Cartier, 1996). We have estimated TSP/HAM prevalence in 1:50 000 inhabitants. The probability of TSP/HAM is about 0.20–0.50% in infected individuals. Mortality has been calculated in 2% per year. Serological studies had showed a relationship between TSP/HAM and the HTLV-I infection in nearly 60% of TSP/HAM patients (Cartier et al., 1989; Galeno et al., 1994). However, approximately a 40% of TSP/HAM patients

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do not seem to be infected with HTLV-I/II because they are HTLV-I/II seronegatives (Cartier et al., 1990; Araujo et al., 1993). These seronegative patients are clinically identical or indistinguishable from TSP/HAM seropositive cases (Cartier et al., 1999).

Previously, we showed the presence of a region of *tax* gene, but not *ltr* gene, in PBMC from ten Chilean seronegative TSP/HAM patients (Ramirez et al., 1998). It is important to note that 40% of these seronegative, *tax*-positive TSP/HAM patients had developed chronic dacriosialoadenitis. This suggests that the development of some forms of Sjögren's syndrome would be associated with the presence of HTLV-I provirus. The goal of this study was to analyze the presence of different genes of the HTLV-I provirus in those Chilean seronegative TSP/HAM patients.

2. Methods

2.1. Patients

We studied sequentially 72 Chilean TSP/HAM patients from the Neurology Service, of 'El Salvador Hospital' (Santiago of Chile). It was completed 40 HTLV-I seronegative and 32 HTLV-I seropositive patients. In all the cases was discarded other causes of spastic paraparesis through the definition of clinical status, biochemical analysis of the CSF, neurophysiologic test (evoked potential), radiological study (mielography and/or Nuclear Magnetic Resonance), immunologic assays (electrophoresis of proteins, antinuclear ENA and ANA antibodies, antireumathoid RF antibodies) and hematological analysis. Clinical diagnoses were made by three different physician belonging to the Neurology Service. All sequential patients were recruited into the study from 1995 until 1999. The studied patients have not any relationship between them.

2.2. Serological assays

Determination of antibodies was accomplished, in sterile and not diluted plasma samples, by enzyme linked immunoassay (ELISA), indirect immunofluorescence assay (IFA) and Western blot (WB). The HTLV-I whole virus ELISA was obtained from Organon Teknika. IFA was performed on slides with HTLV-I infected MT-2 cells according to method previously described (Gallo et al., 1991). The HTLV-I/II WB was obtained from Genelabs Technologies (HTLV Blot 2.4, Singapore). Nitrocellulose strips were derived from an HTLV-I-infected T-cell line (HUT 102) lysate; the strips included the recombinant protein rgp21 (representing a conserved epitope between HTLV-I and HTLV-II), the HTLV-I-specific recombinant protein rpg46-I, and the HTLV-II-specific recombinant protein rpg46-II. WB were performed according to the conditions specified by the manufacturer. Serum was used at dilution of 1:100.

2.3. Polymerase chain reaction gene amplification

DNA was extracted from purified peripheral blood mononuclear cells (PBMC) according to method previously described (Ehrlich et al., 1990). This protocol was performed using 3×10^6 PBMC. An extraction control used identical procedures without PBMC. Protective clothing, separate equipment, newly prepared reagents, ultraviolet irradiation and others measures to prevent contamination were routinely used. We amplified the following genomic regions of HTLV-I: 158 bp of *tax* with primers SK43/SK44, 401 bp of 5′ *ltr* with primers LTR1/LRT6, 332 bp of *env*/*tax* with primers SG452/453, 179 bp of *pX* with primers pX/pX′, 185 bp of *pol* with primers SK110/111 and 251 bp of *env* with primers SG219/294 (Fig. 1). Also, we amplified genomic fragments using the combination of 5′ tax (SK43) with 3′ pX primers (pX′) and 3′ tax (SK44) with 5′ env/tax primers (SG452) (Ehrlich et al., 1990). Primers were used at a final concentration of 1 μM in a reaction mixture of 50 μl containing 1–2 μg of each DNA sample, 2.5 units of *Thermus aquaticus* DNA polymerase (GIBCO BRL, Life Technologies, Inc) and comprised of 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, and 0.2 mM each dNTP. Reaction mixtures were cycled for 35 cycles in a Perkin–Elmer thermal cycler by being heated to 94 °C for 20 s, 50 °C for 20 s, and 72 °C for 20 s. Mixtures was finally heated to 72 °C for 8 min. Laboratory contamination was monitored by parallel analysis of extraction controls lacking PBMC, PBMC from HTLV-I/II seronegative blood donors, and PCR controls without DNA. Amplified DNA for HTLV-I was analyzed by electrophoresis on 7.5% polyacrylmide gel followed by silver nitrate staining. This PCR assays detects as little as two copies of HTLV-I *tax* DNA per 14 000 cells (Ramirez et al., 1998; Villota C., personal communication).

2.4. DNA cloning and sequencing

Amplified products of the tax gene were purified from agarose gels and cloned into the pGEM-T vector (Promega). Nucleotide sequence was determined by the dideoxy termination procedure with the SEQUENASE version 2.0 kit (US Biochemicals). DNA sequences were aligned with the CLUSTAL V program (Higgins et al., 1992).

Primer	Gene	Position *	Sequence	Length of amplified product (bp)
SK43	*tax*	7358-7377	CGGATACCCAGTCTACGTGT	158
SK44	*tax*	7516-7496	GAGCCGATAACGCGTCCATCG	
LTR1	*5`ltr*	10-30	ACCATGAGCCCCAAATATCCC	401
LTR6	*5`ltr*	409-288	GCGTGGATGGCGGCCTCAAGGTA	
SG452	*env/tax*	5796-5818	ATCCTCGAGCCCTCTATACCATG	332
SG453	*env/tax*	6128-6106	GCGGGATCCTAGGGTGGGAACAG	
pX	*pX*	7622-7650	ATGCGCAAATACTCCCCCTTCCGAAATGG	179
pX'	*pX*	7800-7772	AGGAGGGGCCAGGTGATGGGGGGGGAAAG	
SK110	*pol*	4757-4778	CCCTACAATCCAACCAGCTCAG	185
SK111	*pol*	4942-4919	GTGGTGAAGCTGCCATCGGGTTTT	
SG219	*env*	5270-5292	CCCCAGCTGCTGTACTCTCACAA	251
SG294	*env*	5540-5521	TGGGCACTTTAAGGAACAAG	

*: Nucleotide position in ATK-1 clone

Fig. 1. Sequences of synthetic oligonucleotide primers for detection of HTLV-I *tax*, 5′ *ltr*, *env/tax*, *pX*, *pol* and *env* sequence using the PCR method.

2.5. Statistic analysis

Comparisons of epidemiological features between sub-groups of TSP/HAM patients were made by *t* Student's test with EPIDAT2.1 program

3. Results

Seventy-two progressive spastic paraparesis patients composed the studied population. They were 30 men and 42 women. They had an average age of 53.6 years (24–81 years) and the average time of paraparesis was 9.4 years (1–33; Tables 1–3). Both group were identical clinically with only exception of mean time of age of patients: 56.38 ± 11.75 years in seropositives and 50.03 ± 15.46 in seronegatives ($P = 0.0294$).

3.1. Seropositive group

Thirty-two patients were seropositive by ELISA, IFA and WB assays for the presence of antibody to HTLV-I and HTLV-II proteins, respectively. Antibodies to HTLV-I in these samples, as measured by EIA optical density values, were consistently above 2.0, a value well above the 0.45–0.50 seropositivity cutoff (data not shown). Upon confirmation by WB the serum samples from seropositive patients had reactivity against to a conserved HTLV-I/II epitope (rpg21), HTLV-I specific env recombinant glycoprotein (rgp46-1) and gag antigens (p19 and p24). Seven men and 25 women composed the seropositive group. They presented by definition a progressive spastic paraparesis (Tables 1 and 3). Almost 50% of them showed gait without support, five (16%) patients no had gait and 1/3 needed support to walk. The average time of disease evolution was 9.6 years. Radiological studies showed spinal cord atrophy in 60% of the patients. Electrophysiological studies showed involvement of SSEP in 80% of patients, only seven cases (22%) had some clinical manifestations of the sensitive deficit. Eighty percent of patients had Sjögren's syndrome associated to HTLV-I, established by Schirmer test and biopsy of minor salivary glands.

3.2. Seronegative group

Forty patients were HTLV-I/II seronegative by IFA, ELISA and WB tests. Antibodies to HTLV-I in these samples, as measured by EIA optical density values, were consistently lower than the seropositivity cutoff. The samples of seronegative patients lacked antibodies to any HTLV-I/II antigens by WB. We did not detect

Table 1
Clinical features of 32 HTLV-I-seropositive Chilean patients with TSP/HAM

Patient number	Age (years)	Sex	Evolution (years)	Functionality	Sensitive involvement	Associated complex	PCR *tax*
1	64	M	8	Gait without support	SSEP altered	Chronic ATL Sjögren's syndrome	+
2	65	F	9	Gait without support	None	Sjögren's syndrome	+
3	57	F	15	Gait with support	SSEP altered	None	+
4	70	M	10	Gait without support	SSEP altered	Sjögren's syndrome, cutaneous lesions	+
5	58	F	3	Gait without support	None	None	+
6	60	M	14	Gait without support	SSEP altered	Sjögren's syndrome, diabetes	+
7	56	F	5	Gait without support	SSEP altered	Sjögren's syndrome, cutaneous lesions	+
8	59	F	5	Wheelchair	Hypoesthesia	Sjögren's syndrome	+
9	73	F	28	Wheelchair	Hypoesthesia	Sjögren's syndrome, bronchoalveolitis	+
10	69	F	14	Gait with support	Hypoesthesia	Sjögren's syndrome	+
11	66	F	9	Gait with support	SSEP altered	Sjögren's syndrome, bronchoalveolitis	+
12	30	F	9	Gait without support	SSEP altered	Sjögren's syndrome	+
13	39	M	7	Gait without support	None	Sjögren's syndrome	+
14	63	F	9	Gait without support	SSEP altered	Sjögren's syndrome	+
15	60	F	5	Gait with support	SSEP altered	Sjögren's syndrome	+
16	65	F	15	Gait with support	SSEP altered	Sjögren's syndrome	+
17	39	F	4	Gait without support	SSEP altered	Sjögren's syndrome, arthralgias	+
18	60	F	3	Gait with support	SSEP altered	Sjögren's syndrome	+
19	52	F	5	Gait with support	SSEP altered	Sjögren's syndrome	+
20	28	F	4	Gait without support	SSEP altered	None	+
21	71	F	8	Gait with support	SSEP altered	Sjögren's syndrome	+
22	58	F	10	Gait without support	SSEP altered	Sjögren's syndrome, cutaneous lesions	+
23	68	F	12	Gait with support	Hypoesthesia	Sjögren's syndrome, diabetes	+
24	42	M	4	Wheelchair	Hypoesthesia	Cutaneous lesions	+
25	39	M	18	Wheelchair	Hypoesthesia	Chronic ATL, Sjögren's syndrome	+
26	59	F	12	Gait without support	None	Sjögren's syndrome	+
27	57	F	33	Gait without support	SSEP altered	Sjögren's syndrome	+
28	43	F	5	Gait without support	None	None	+
29	56	F	3	Wheelchair	SSEP altered	Sjögren's syndrome	+
30	66	M	6	Gait with support	SSEP altered	Sjögren's syndrome, arthralgias	+
31	56	F	3	Gait without support	Hypoesthesia	None	+
32	56	F	10	Gait without support	None	None	+

SSEP, somato-sensory evoked potentials; ATL, adult T-cell leukemia.

any serum sample with HTLV-I/II seroindeterminate WB profile. Furthermore, selection of these seronegative patients was based on the absence of any reactivity against HTLV-I/II by WB assay. Twenty-two men and 18 women composed the seronegative group, and all of them have a progressive spastic paraparesis. Sixty-eight percent of them showed gait without support; 7.3% of patients had no gait and 27.3% needed support to walk. The average time of disease evolution in this group was 10 years. Between 17 tax positive cases by PCR, the neuroradiological finding show 52.9% with spinal cord atrophy (Tables 2 and 3). Electrophysiological analysis

Table 2
Clinical features of 40 HTLV-I-seronegative Chilean patients with TSP/HAM

Patient number	Age (years)	Sex	Evolution (years)	Functionality	Sensitive involvement	Associated complex	PCR *tax*
1	44	F	4	Gait without support	SSEP altered	Hepatic cirrhosis, Sjögren's syndrome	+
2	64	F	15	Wheelchair	Hypoesthesia	Sjögren's syndrome	−
3	45	M	2	Gait without support	None	None	−
4	32	F	2	Gait without support	Hypoesthesia	None	−
5	81	M	10	Wheelchair	None	None	+
6	74	F	8	Gait without support	SSEP altered	None	−
7	30	F	2	Gait with support	None	None	+
8	54	M	14	Gait with support	Hypoesthesia	None	+
9	57	F	5	Gait without support	Hypoesthesia	Sjögren's syndrome	+
10	46	M	3	Gait without support	None	Cutaneous lesions	+
11	73	F	3	Gait with support	SSEP altered	Sjögren's syndrome	+
12	52	F	1	Normal Gait	None	Hepatic cirrhosis	+
13	46	M	7	Gait without support	None	None	−
14	58	M	2	Gait without support	Hypoesthesia	None	+
15	71	F	31	Gait without support	Hypoesthesia	Sjögren's syndrome	−
16	63	F	22	Gait without support	SSEP altered	Sjögren's syndrome/cutaneous lesions	+
17	32	M	7	Gait without support	None	None	−
18	47	F	20	Gait without support	None	None	−
19	48	M	18	Gait without support	SSEP altered	None	−
20	56	M	24	Gait with support	Hypoesthesia	Sjögren's syndrome	−
21	30	M	2	Gait without support	Hypoesthesia	None	+
22	58	M	2	Gait without support	None	None	−
23	38	F	10	Gait without support	None	None	+
24	36	F	20	Gait without support	None	None	−
25	42	F	9	Gait without support	Hypoesthesia	None	−
26	59	F	7	Gait without support	SSEP altered	None	+
27	50	M	4	Gait without support	SSEP altered	None	−
28	58	M	22	Gait without support	Hypoesthesia	None	−
29	47	F	2	Gait without support	None	None	−
30	64	M	23	Gait without support	None	None	−
31	41	M	8	Gait without support	SSEP altered	None	−
32	69	M	2	Gait with support	Hypoesthesia	Sjögren's syndrome	+
33	55	M	22	Wheelchair	None	None	−
34	65	F	25	Gait without support	None	None	−
35	60	F	25	Gait with support	SSEP altered	None	−
36	51	M	3	Gait with support	None	None	−
37	24	M	2	Gait without support	SSEP altered	None	+
38	62	M	19	Gait with support	SSEP altered	None	+
39	27	M	3	Gait without support	None	None	−
40	42	M	6	Gait with support	SSEP altered	Sjögren's syndrome	+

SSEP, somato-sensory evoked potentials.

showed 58% with SSEP involvement, and 25% had some clinical manifestations of sensitivity. Thirty-five percent of tax positive patients by PCR showed Sjögren's syndrome and 23% of them had cutaneous lesions. Thirty-three patients did not show any evidence for to be associated to HTLV-I infection.

3.3. DNA study

DNA was prepared from PBMC from each seropositive patient in order to confirm that these patients were infected with HTLV-I. We studied the presence of HTLV-I provirus in PBMC from 32 seropositive TSP/HAM patients by PCR. The regions of the HTLV-I genome that were amplified are shown in Fig. 1. We detected amplified products of 5′ *ltr*, *tax*, *env*/*tax*, *pX*, *pol* and *env* genes in all of these patients. The specificity of the HTLV-I gene amplifications by PCR were confirmed by nucleotide sequencing of amplified products. The HTLV-I *tax*, 5′ *ltr*, *gag*, *pol*, and *env* gene were found to be 99.6, 96.3, 99.4, 96.5 and 96.7% homologous to prototypic HTLV-I provirus from ATK-1 clone, respectively (submitted).

We studied the presence of HTLV-I provirus in PBMC from the 40 seronegative patients by PCR in order to discard definitively the viral infection in these

Table 3
Summary of clinical features of 32 seropositive and 40 HTLV-I-seronegative Chilean patients with TSP/HAM

Type of patient	Mean age (years)	Sex		Mean evolution (years)	Functionality	Sensitive involvement	Associated complex
		M	F				
Seropositive	56.38±11.75	7	25	9.53±6.87	Seventeen gait without support Ten gait with support Five wheelchair	Nineteen SSEP altered Seven hypoesthesia Six none	Twenty-five Sjögren's syndrome
Seronegative	50.03±15.46	22	20	10.01±8.00	Twenty-seven gait without support	Twelve SSEP altered	Nine Sjögren's syndrome
	$P = 0.0294$			$P = 0.39436$	Nine gait with support Three wheelchair One normal Gait	Eleven hypoesthesia Seventeen none	

SSEP, somato-sensory evoked potentials.

cases. We analyzed the same profile of genomic regions that seropositive patients (Fig. 1). All seronegative patients were negative with 5′ *ltr* and *pX*, *pol* and *env* primers. However, amplified product of *tax* gene was detected from 16 seronegative patients, four cases was positive with primers of *env*/*tax* region (Fig. 2). Three of them were simultaneously positive with *tax* and *env*/*tax* genetic regions. The specificity of the HTLV-I *tax* gene amplification by PCR was confirmed in six seronegative patients by nucleotide sequencing of amplified products (Fig. 3). The nucleotide sequence from six seronegative patients showed a great homology with ATK-1 clone, ranged from 98.7 to 99.4%. The provirus of patient 38 had one nucleotide mutation at position 7416 (T/A), but it did not produce any substitution on amino acid sequence. The provirus of patient 5 had two mutations at positions 7358 (G/A) and 7475 (G/C), both of them produced changes of amino acids: glycine to arginine

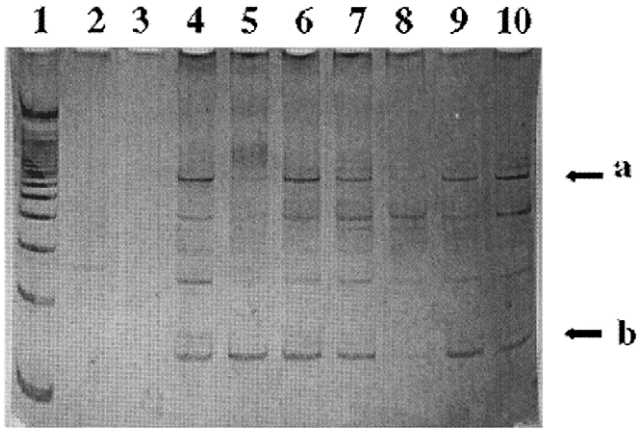

Fig. 2. Detection of *tax* gene by polymerase chain reaction. 1, molecular weight marker (100 bp ladder); 2, negative control (distilled water); 3. Negative control (H9 cells); 4, positive control (MT2 cells); 5, patient number 1 (tax positive); 6, patient number 2 (tax positive); 7, patient number 3 (tax positive); 8, patient number 4 (tax negative); patient number 5 (tax positive); 10, patient number 6 (tax positive). Arrows: a, internal control (fragment of β-globin 398 bp); b, tax gene (fragment of 158 bp).

(Gly/Arg) and isoleucine to threonine (Ile/Thr), respectively. The provirus of patient 26 had only one nucleotide mutation at position 7348 (C/A); it did not generate any amino acid change. The proviruses of patients 8, 9, and 11 had two identical mutations, at positions 7358 (G/A) and 7449 (A/T). Only one of these mutations, at position 7358, caused a change of glycine to arginine (Gly/Arg). Table 2 describes the clinical findings of the 17 HTLV-I seronegative patients that show the presence of an amplified product of tax or env/tax gene. They were eight men and nine women. They had an average age of 55.9 years (30–81 years) and the average time of paraparesis was 6.5 years (1–22 years). Six of them presented dacriosialoadenitis as associated pathology).

Several TSP/HAM seronegative patients were only positive by PCR with tax and/or tax/env primers showing amplified fragments of 158 and 332 bp, respectively. Because these amplified products had a small length we wanted to amplify greater length fragments of tax gene. We made PCR assays using 5′ tax (SK43) with 3′ pX primers (pX′) and 3′ tax (SK44) with 5′ env/tax primers (SG452). The combination of SK43 with pX′ and SK44 with SG452 primers would generate a fragment of 454 and 1744 bp, respectively. All PCR with DNA from seronegative patients were negative using these combinations of primers (results not shown). By serial dilutions of DNA samples we detected amplified product of *tax* by PCR using as little as 1–2 μg of each DNA sample in seronegative patients. However, it was possible to detect amplified product of *tax* in seropositives patients using until 10^3–10^4 dilutions of original DNA sample (1–2 μg; results not shown).

4. Discussion

In the present study, we confirm the viral infection in PBMC from all seropositive patients because we detected amplified products of 5′ *ltr*, *tax*, *evn*/*tax*, *pX*, *pol*

```
ATK-1       7336CGGATACCCAGTCTACGTGTTTGGAGACTGTGTACAAGGCGACTGG7381
Patient 5       ......................A......................
Patient 8       ......................A......................
Patient 9       ......................A......................
Patient 11      ......................A......................
Patient 26      ............A.............................
Patient 38      ........................................

ATK-1       7382TGCCCCATCTCTGGGGGACTATGTTCGGCCCGCCTACATCGTCACG7427
Patient 5       ........................................
Patient 8       ........................................
Patient 9       ........................................
Patient 11      ........................................
Patient 26      ........................................
Patient 38      ................................A.......

ATK-1       7428CCCTACTGGCCACCTGTCCAGAGCATCAGATCACCTGGGACCCCAT7473
Patient 5       ........................................
Patient 8       ......................T.................
Patient 9       ......................T.................
Patient 11      ......................T.................
Patient 26      ........................................
Patient 38      ........................................

ATK-1       7474CGATGGACGCGTTATCGGCTC7494
Patient 5       .C..................
Patient 8       ....................
Patient 9       ....................
Patient 11      ....................
Patient 26      ....................
Patient 38      ....................
```

Fig. 3. Nucleotide sequence of 158 bp of the *tax* gene from six HTLV-I-seronegative patients with TSP/HAM. The *tax* region of six Chilean TSP/HAM and ATK-1 proviruses were amplified with SK43 and SK44 primers.

and *env* genes in PBMC by PCR. Also, we defined the viral source of these amplified fragments through analysis of nucleotide sequence of amplified product of tax gene from TSP/HAM seropositive patients (Ramirez, Cartier, Fernandez and Villota, unpublished results). These results suggest the presence of a complete HTLV-I provirus in all seropositive Chilean patients with TSP/HAM-HAM. However, we cannot discard the possible presence of some defective HTLV-I provirus in those patients. Kira et al. found frequently pX-defective mutants of HTLV-I in frozen central nervous system tissue in patients with TSP/HAM (Kira et al., 1994). They postulated that the presence of this incomplete provirus might contribute to the neural damage, since the pX gene products are essential for the transactivation of various cellular genes as well as for viral replication.

We had previously reported the presence of tax gene in the PBMC of seronegative TSP/HAM. Also, we showed a nucleotide homology of amplified product of tax gene from seronegative patients ranged between 98.7 and 99.4% to ATK-1 clone (Ramirez et al., 1998; Galeno et al., 1996). However, in those studies we did not able to determine the length of proviral present in these patients because it was analyzed only two genetic regions of HTLV-I provirus, tax and 5′ ltr. Now in this study, we analyzed by PCR six different regions of viral genome from structural and regulatory genes. We have showed the presence of a truncated genome of HTLV-I in PBMC of the seronegative TSP/HAM because we detected *tax* gene in 17 of 40 of those TSP/HAM patients. The specificity of the HTLV-I *tax* gene in six patients was confirmed by nucleotide sequencing of the PCR products showing. This analysis showed that tax region of seronegative patients was 98.9% homologous with ATK-1 clone. We could not detect any other viral gene in these individuals. We could amplify *tax* using as little as 1–2 μg of each DNA sample from seronegative patients. However, amplified product of *tax* from seropositives was detected using until 10^3–10^4 dilutions of original DNA sample (1–2 μg). These results suggested that the genetic load in PBMC from seropositive patients is higher than seronegatives. Despite of we have detected an amplified a fragment of 158 bp of tax with SK43/44 primers it was not possible to define any bigger region of this gene with different combinations of primers from 3′ and 5′ nested terminal. We think that there are two hypotheses to explain this result. First, our PCR assay had a methodological problem to amplify this new fragment from seronegative samples. However, these combinations of primers are able to detect flanking regions from positive control. Other reason to explain this finding is the absence of sequences of primers outer *tax* gene in nucleotide sequence from DNA target from seronegative patients.

The exclusive presence of *tax* gene has been reported in some cutaneous T-cell lymphoma and Sjögren's syndrome patients. Ghosh demonstrated the presence of *tax* gene but not *gag* gene in cutaneous T-cell lymphoma from 72% (18/25) of Sesary syndrome patients, suggesting a probably relationship between the defective provirus in the pathogenesis (Ghosh et al., 1994). Nagato and Sumida also have detected only the HTLV-I *tax* gene but not the *gag*, *pol*, or *env* gene in labial salivary gland samples from 29% (4/14) Sjögren's syndrome (SS) patients (Yonaha-Nagato and Sumida, 1995). We have previously described the relationship between SS, TSP/HAM and infection with HTLV-I (Cartier et al., 1995). Previously, we had found Sjögren's syndrome in 66.7% of seronegative *tax* positive TSP/HAM patients (Cartier et al., 1999). Now, we have reported Sjögren's syndrome in 35% of this kind of patients. On the other hand, Castro Costa did not detect any *tax*/*rex* or *pol* region in PBMC from 12 seronegative TSP/HAM patients from Brazil (de Castro Costa et al., 1995). However, Nishimura detected HTLV-I *pol* and *tax* viral sequences in DNA from fresh peripheral blood lymphocytes (PBL) from an HTLV-I seronegative Indian patient with TSP/HAM by PCR and liquid hybridization techniques (Nishimura et al., 1993). A long-term CD4+ T-cell line was established from PBL of this patient. DNA from these cultured lines was amplified and portion of *ltr*, *pol*, *env*, and *tax* were sequences. The sequence data showed that the HTLV-I associated with this patient was 98.8% homologous to prototype HTLV-I. Recently, Koya described the establishment of a seronegative HTLV-I carrier state in rats inoculated with HTLV-I-infected rat T cell line, FPM1, which preferentially express HTLV-I Tax (Koya et al., 1999). The main reason for the negative antibody responses in inoculated rats would be the extremely low amounts of HTLV-I antigens in those infected cells.

In summary, these results showed the presence of a fragment of HTLV-I provirus in 42.5% of seronegative TSP/HAM patients. This finding suggesting 40% of these seronegative TSP/HAM patients could be infected with an extremely low amount of HTLV-I. The study of these patients could be very interesting to understand the relationship of tax gene on the development of spastic paraparesis.

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