JOURNAL OF CLINICAL MICROBIOLOGY, June 1998, p. 1811–1813 Vol. 36, No. 6
0095-1137/98/$04.00+0

Defective Human T-Cell Lymphotropic Virus Type I (HTLV-I) Provirus in 10 Chilean Seronegative Patients with Tropical Spastic Paraparesis or HTLV-I-Associated Myelopathy

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Received 22 August 1997/Returned for modification 12 November 1997/Accepted 23 March 1998

We studied the presence of *tax* and *ltr* genes from human T-cell lymphotropic virus type I (HTLV-I) provirus in the peripheral blood mononuclear cells from 15 seronegative patients with tropical spastic paraparesis or HTLV-I-associated myelopathy by PCR. Only a region of the *tax* gene from 10 patients was amplified. The nucleotide homologies of six Chilean isolates to the ATK-1 clone ranged between 98.7 and 99.4%.

Infection with human T-cell lymphotropic virus type I (HTLV-I) has been associated with the development of tropical spastic paraparesis or HTLV-I-associated myelopathy (TSP-HAM) (9, 17). Chilean studies have shown that almost 50% of patients with progressive spastic paraparesis (PSP) are HTLV-I seropositive (1). Diagnosis of this viral infection is done mainly by detection of specific antibodies (2, 7). The PCR assay has permitted detection of the provirus in peripheral blood mononuclear cells (PBMC) (5). Recently, some researchers have reported seronegative patients with PSP who are infected with HTLV-I (6, 16).

Seropositive and seronegative patients with PSP are clinically indistinguishable. A clinical study of these groups showed that seronegative patients had poor inflammatory response in their cerebrospinal fluid (CSF), absence of leukemoid lymphocytes in their peripheral blood, and less neurophysiologic involvement in the study of somatosensorial evoked potentials (1).

We studied 15 HTLV-I-seronegative patients with PSP (7 men and 8 women). They had an average age of 55.1 years (40 to 74 years) and an average paraparesis duration of 7.4 years (2 to 20 years). Other causes of PSP were excluded through clinical presentation according to cytochemical analysis of CSF and neurophysiological, radiological, immunological, and hematological analyses.

All patients had PSP with spasticity, hyperreflexia, and weakness of lower limbs; bilateral Babinski signs; and some sphincter disturbance. In addition to the spastic paraparesis, seven patients had brain involvement. Four of these developed pseudobulbar signs (dysartria, dysphagia, and affective lability). Three patients had basal ganglion involvement (two developed a Parkinsonian syndrome, and one showed diskinetic movements). Four patients had dacryosialadenitis, which was diagnosed by Schirmer's test and by biopsies of minor salivary glands (3). Clinical data for each patient are presented in Table 1.

Determination of antibodies was accomplished by indirect immunofluorescence assay and Western blotting (WB) (8). DNA was extracted from purified PBMC according to a previously described method (5). By PCR, we amplified a region of 158 bp (primers SK43 to -44) of the *tax* gene and a region of 401 bp (primers LTR1 and LTR6) of the *ltr* gene (5). In six patients, 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 (U.S. Biochemicals). DNA sequences were aligned with the CLUSTAL V program (12).

All 15 patients were HTLV-I seronegative by indirect immunofluorescence and WB assays. Furthermore, all cases were negative for anti-p40 Tax antibodies by WB.

The *tax* gene was amplified from PBMC of 10 patients (5 men and 5 women), and the *ltr* gene was not detected in any of these patients. These results were confirmed through analysis of sequential samples from 6 patients (Table 1).

Sequences obtained from Chilean patients were compared with that of the HTLV-I prototype clone ATK-1 (Fig. 1). The *tax* sequences of patients 2, 4, 5, 7, 8, and 10 showed 99.4, 98.7, 99.4, 98.7, 98.7, and 98.7% homology, respectively, to the nucleotide sequence of the ATK-1 clone. Nucleotide homologies among samples in this region were 100% (patients 7, 8, and 10), 99.4% (patients 7, 8, and 10 compared to patient 4), 98.7% (patients 7, 8, and 10 compared to patients 2 and 5), 98.1% (patient 4 compared to patients 2 and 5), and 98.7% (patient 2 compared to patient 5). The nucleotide sequences of patients 4, 7, 8, and 10 had two nucleotide changes compared to the sequence of ATK-1; those of patients 2 and 5 had one nucleotide change. One of the nucleotide changes (nucleotide 7380) was common to four patients, and another (nucleotide 7469) was common to three patients (Table 2).

We detected the *tax* gene in 10 of 15 HTLV-I-seronegative patients with PSP using PCR analysis. However, we did not detect the *ltr* gene in any of these patients. These results could not be explained by a different sensitivity in the genetic amplification method in different genomic regions of HTLV-I, because *tax* and *ltr* amplifications had similar sensitivities (6, 7).

Another hypothesis to explain our results is the presence of defective provirus in the PBMC from these patients. Yonaha-Nagato and Sumida found only the HTLV-I *tax* gene but not the *gag*, *pol*, or *env* gene in labial salivary gland samples from 29% of patients with Sjögren's syndrome (23). Others researchers demonstrated the presence of a truncated HTLV-I genome from 72% of patients with Sezary's syndrome (11). Our results showed the detection of *tax* but did not confirm *ltr* in 10 patients with TSP-HAM. These results support the hy-

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TABLE 1. Clinical features of 15 HTLV-I-seronegative Chilean patients with PSP

Patient no.	Sex	Age (yrs)	Evolution time (yrs)	Motor involvement	Sensitive symptom(s)	Spastic gait	Bladder involvement	Associated pathology	*tax* PCR
1	F	62	13	Pseudobulbar signs and tetraparesis	Impairment in lower limbs and paresthesia	With support	Urgency[a]	None	Positive
2	M	46	10	Paraparesis	Vibratory sense impairment of lower limbs	Independent	Frequency[b]	None	Positive
3	F	61	20	Paraparesis and Parkinsonian signs	Vibratory sense impairment of lower limbs and paresthesia	Independent	Frequency	Dacryosialadenitis	Positive
4	M	74	5	Paraparesis	None	With support	Frequency	None	Positive
5	F	55	2	Paraparesis and diskinesis	None	Independent	Frequency	Cutaneous lesions	Positive
6	M	40	4	Pseudobulbar signs and tetraparesis	None	With support	Urgency	Dacryosialadenitis	Positive
7	F	48	3	Pseudobulbar signs and tetraparesis	Tactile and vibratory sense impairment in lower limbs and paresthesia	Bedridden	Urgency and incontinence[c]	Dacryosialadenitis and hepatic cirrhosis	Positive
8	M	73	4	Paraparesis	None	Independent	Normal	Multiple neurofibramatosis	Positive
9	F	42	3	Paraparesis and Parkinsonian syndrome	Impairment in lower limbs and paresthesia	Independent	Frequency	Dacryosialadenitis and cutaneous lesions	Positive
10	M	50	10	Pseudobulbar signs and tetraparesis	None	Independent	Frequency	None	Positive
11	F	70	6	Paraparesis	None	Independent	Frequency	None	Negative
12	M	52	12	Paraparesis	Arm pain	With support	Normal	None	Negative
13	F	30	1	Paraparesis	None	Independent	Normal	None	Negative
14	M	60	17	Paraparesis	None	With support	Frequency	None	Negative
15	F	56	4	Paraparesis	Impairment in lower limbs and paresthesia	Independent	Normal	None	Negative

[a] Urinary urgency.
[b] Urinary urgency and occasional urinary oncontinence.
[c] Urinary frequency.

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         7358                                          7403
ATK      CGGATACCCAGTCTACGTGTTTGGAGACTGTGTACAAGGCGACTGG
CR-1     ....................................G.........
Pac 2    ..............................................
Pac 4    ......................A.......................
Pac 5    .............A................................
Pac 7    ......................A.......................
Pac 8    ......................A.......................
Pac 10   ......................A.......................

         7404                                          7449
ATK      TGCCCCATCTCTGGGGGACTATGTTCGGCCCGCCTACATCGTCACG
CR-1     ................................T.............
Pac 2    ..................................C...........
Pac 4    ..............................................
Pac 5    ..............................................
Pac 7    ..............................................
Pac 8    ..............................................
Pac 10   ..............................................

         7450                                          7495
ATK      CCCTACTGGCCACCTGTCCAGAGCATCAGATCACCTGGGACCCCAT
CR-1     ..............................................
Pac 2    ..............................................
Pac 4    .............................................C
Pac 5    ..............................................
Pac 7    ...................T..........................
Pac 8    ...................T..........................
Pac 10   ...................T..........................

         7496                 7516
ATK      CGATGGACGCGTTATCGGCTC
CR-1     .....................
Pac 2    .....................
Pac 4    .....................
Pac 5    .....................
Pac 7    .....................
Pac 8    .....................
Pac 10   .....................
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FIG. 1. Nucleotide sequence of 158 bp of the *tax* gene from six Chilean HTLV-I-seronegative patients with PSP.

pothesis of an incomplete presence of the provirus in the PBMC of these seronegative TSP-HAM patients.

On the other hand, it is important to note that 40% of these seronegative, *tax*-positive TSP-HAM patients had developed chronic dacriosialoadenitis. This finding suggests that the development of some forms of Sjögren's syndrome would be associated with the presence of HTLV-I provirus (3, 23).

Seropositive patients with TSP-HAM have high levels of antibodies against HTLV-I (1, 13, 15). However, our 10 *tax*-positive patients with TSP-HAM did not have antibodies against HTLV-I. This finding would discard an immune-inflammatory process as a pathogenic mechanism in these patients (20). Others researchers have suggested that antigens of HTLV or products encoding sequences homologous to the HTLV-I genes in PBMC from patients with TSP-HAM might

TABLE 2. Nucleotide sequence homology of 158 bp of the *tax* gene between the ATK-1 clone and six HTLV-I-positive Chilean isolates

Isolate	% Homology						
	ATK-1	Isolate 2	Isolate 4	Isolate 5	Isolate 7	Isolate 8	Isolate 10
ATK-1		99.4	98.7	99.4	98.7	98.7	98.7
2	99.4		98.1	98.7	98.7	98.7	98.7
4	98.7	98.1		98.1	99.4	99.4	99.4
5	99.4	98.7	98.1		98.7	98.7	98.7
7	98.7	98.7	99.4	98.7		100	100
8	98.7	98.7	99.4	98.7	100		100
10	98.7	98.7	99.4	98.7	100	100	

be candidates for self-antigen and/or might lead to activation of autoreactive T lymphocytes by an immunoglobulin-mediated mechanism (19, 22). However, for our patients it seems more plausible to explain the pathogenesis by degenerative lesions of the central nervous system (21). We think that HTLV-I infection, in relation to genetic condition, has a direct effect on the development of spastic paraparesis.

HTLV-I is genetically very stable. A low degree of genetic variation (0.5 to 3%) has been described for HTLV-I strains from Japan, Africa, the Caribbean basin, and the Americas (4, 10, 14, 18). The *tax* fragment from our patients showed 98.7 to 99.4% homology with that of the ATK-1 genetic sequence. These findings showed that the amplified fragment is highly homologous to HTLV-I provirus sequence. In addition, the fact that some mutations in the *tax* gene were present in patients 3 and 4 suggests that the level of random mutation due to errors in *Taq* polymerase was not responsible for the overall differences observed. In summary, the results of this study suggest the presence of a defective HTLV-I provirus in these seronegative patients with TSP-HAM.

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