No evidence of HTLV-I infection in patients with mycosis fungoides and Sezary syndrome

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The involvement of human T-cell lymphotropic virus type I (HTLV-I) in the etiology of cutaneous T-cell lymphomas (CTCL) is still controversial. The aim of the study was to evaluate the role of HTLV-I in the pathogenesis of mycosis fungoides (MF) and Sezary syndrome (SS) in Polish patients.

The studied group consisted of 42 patients with MF, 5 with SS and 25 with chronic dermatitis. DNA was extracted from snap-frozen and paraffin-embedded skin biopsies and from peripheral blood. Polymerase chain reaction (PCR or nested PCR) was carried out for amplification of different regions of HTLV-I genome. Primer sets flanking pX, p 19, U5, tax and pol genes were used in the investigation. The presence of HTLV-I antibody was examined in 46 sera samples with the use of anti-HTLV-I/II EIA test.

HTLV-I antibodies were not detected in any collected sera samples. PCR with two primer sets homologous to the pX region of HTLV-I showed negative results in all samples investigated. To confirm these results two other primer pairs specific for U5 and gag regions were designed. With these primer pairs no PCR product, except that in positive control, was observed. For more sensitive amplification a nested-PCR with pol and tax specific primers was performed.

HTLV-I probably does not play an important role in the pathogenesis of MF in Polish patients.

Key words: HTLV-I infection, mycosis fungoides, Sezary syndrome

Mycosis fungoides (MF) and Sezary Syndrome (SS) are the most common forms of primary cutaneous T-cell lymphomas (CTCL) [18]. The etiology of the diseases still remains unknown [14]. Some environmental and occupational exposures have been implicated as a possible cause of CTCL, but subsequent case-control studies have failed to support this hypothesis [16]. Human T-cell lymphotropic virus type-I (HTLV-I), an etiological factor of adult T-cell leukemia/lymphoma has been investigated as a possible cause of MF since the similarity between the skin lesions of MF and SS and those of adult T-cell lymphoma /leukemia suggested a possible T-retroviral role in CTCL [5, 20]. Some authors have suggested that a variant or defective type of HTLV-I may play a pathogenic role in the development of the disease, whereas others have been unable to detect HTLV-I proviral sequences in peripheral blood mononuclear cells and lesions from patients with MF or SS [7, 21]. However, since antibodies to HTLV-I/II are only rarely found in sera of patients with MF or SS, the precise role of HTLV-I in the pathogenesis of CTCL is still uncertain [1, 3, 5, 13, 20, 21].

The aim of the study was to estimate the role of HTLV-I in the etiology of MF and SS within the population of Polish patients.

Since no epidemiological data for the prevalence of MF and different types of CTCL is available, the authors estimate (based on reported prevalence for other countries) that some 250–300 cases of MF would have occurred within the study period. Our sample of 42 patients is therefore statistically representative of the Polish patient population.

Material and methods

The studied group consisted of 42 patients with MF and 5 with SS treated at the Department of Dermatology and Oncology, Poznan, Poland during the time period 1994–2000. The diagnosis was established by clinical observation

Table 1. Primer sequences used for identification of HTLV-I DNA

Amplified region	Primer	5'-3'Sequence	Reaction	Product size (bp)	References
pX	pX1	CCTCCGTCAGCTACGACAC			
	pX2	GGAGCGCCGTGAGCGCAAG	PCR	317	[12]
Tax/rex	tax/rex 1	GTTCGGCCCGTCTACATCGTCA			
	tax/rex 2	GGGTGGTGGGCAAACAGTCCTC	PCR	543	
U5	1U5	GCCATCCACGCCGGTTGAGTCG			
	2U5	AGGGCTAGCGCTACGGGAAAAGAT	PCR	438	
p19	p19a	CACCCCTTTCCCTTTCATTCACGA			
	p19b	CCGGCCGGGGTATCCTTTT	PCR	411	
Pol	HL110	CAAGCCTAGCTACATAAAC	Nested PCR		
	HL111	GCGGCTATTAAGACCAGGAAG	(1. round)		[13]
Pol	SK110-2	TCCCCTACAATCCAACCAGCTC	Nested PCR		
	SK111	ATGGGTTTGTTTATTGCTGAGGG	(2. round)	188	[13]
Tax	HL43	ATGCTTATTATCAGCCCACTT	Nested PCR		
	HL44	AGGGTCTTAGAGGTTCTCTGGGT	(1. round)		[13]
Tax	SK43	CCAGTCTACGTGTTTGGAGA	Nested PCR		
	SK44	AGCCGATAACGCGTCCATCGA	(2. round)	159	[13]

and confirmed by routine laboratory examinations, histopathological and immunohistochemical analysis of skin biopsies and lymph node biopsies in cases with lymphadenopathy. Twenty five patients suffering from chronic dermatitis were also investigated as a control group. None of patients of both groups were defined as at high risk of HTLV-I infection.

Amplification of different regions of HTLV-I genome was carried out using polymerase chain reaction (PCR or nested PCR) with specific set of primers (Tab. 1). DNA was extracted from paraffin-embedded skin biopsies of all the investigated patients using the method of deparaffinization for 30 minutes in xylene, as described [11]. Blood samples, fresh or snap-frozen tissue were also available from 23 patients with MF, 5 with SS and 10 with chronic dermatitis. DNA was extracted using QIAamp DNA Mini Kit (Qiagen) with a standard QIAamp tissue protocol or QIAamp Blood DNA Mini Kit (Qiagen). The specimens were then amplified with β-actin primers in order to confirm PCR-suitable DNA. Seven primer sets flanking different region of HTLV-I DNA were used. The location of different primer pairs homologous to HTLV-I DNA sequence is presented in Figure 1. 50–100 ng of DNA was subjected to PCR or nested PCR testing. The reaction mixture contained 0.4 U of Tag polymerase (Polygene), 1 μM of each primer, 200 μM each of deoxynucleotide triphosphate (Boeringer), 1x PCR buffer in a final volume of 10 μl. For nested PCR 2 μl of "first round"-product was subjected to the "second round". Amplification was carried out by 5 minutes denaturation at 95 ˚C, followed by 30 cycles of 30 seconds denaturation at 95 ˚C, 1 minute annealing at 60 ˚C for each primer set excluding p19a/b and pX 1/2. These primers were annealed at 66 ˚C and 56 ˚C, respectively. Following annealing a 1 minute extension at 72 ˚C was performed. The final elongation was increased to 7 min. For nested PCR the samples were subjected to only 25 cycles in each round. PCR products were separated on 2% agarose gel or 5% PAA gel and then stained with 50 μg/ml ethidium bromide. Some of the PCR products of the HTLV-I-pX region amplification were purified using the QIAquick PCR Purification Kit (Qiagen) and some sequenced on an automated DNA sequencer by the Taq cycle sequencing method with pX.

Forty-six sera samples (41 from CTCL patients and 5 from chronic dermatitis) were examined for the presence of HTLV-I antibodies using Cobas Core anti-HTLV-I/II EIA test (Roche), based on recombinant proteins consisting of HTLV-I envelope antigens.

Results

The EIA test for anti-HTLV-I antibodies gave negative results in all collected sera samples. Using PCR with two primer sets homologous to the pX region of HTLV-I (primers: pX1/pX2 and tax/rex1/tax/rex2), negative results were obtained for all investigated samples, both from the studied patients and the control group.

In order to confirm these results two other primer pairs

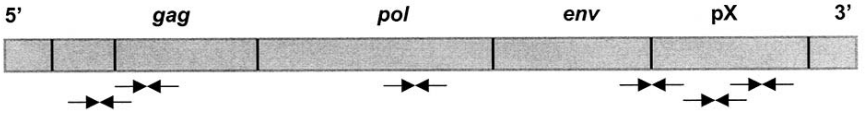

Figure 1. Location of different primer pairs homologous to HTLV-I sequence.

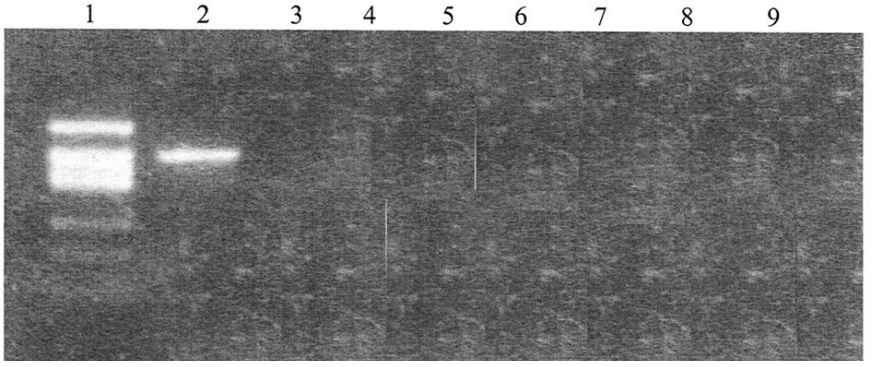

Figure 2. Identification of p19 region of HTLV-I DNA by PCR. 1 – molecular weight markers; 2 – positive control, 3–9 skin biopsies from patients suffering from mycosis fungoides.

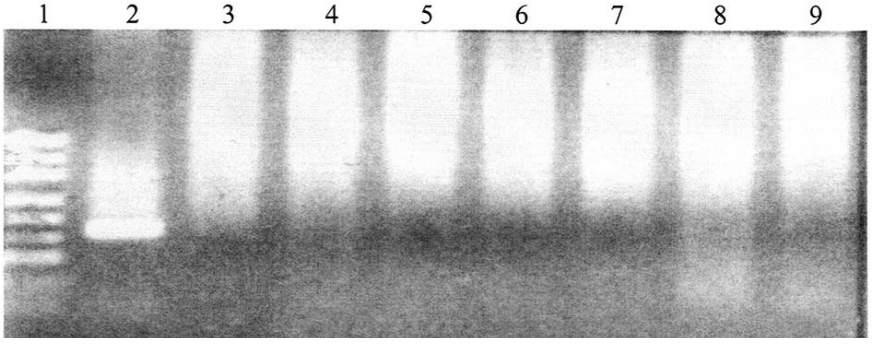

Figure 3. Nested PCR specific for pol region; 1 – molecular weight markers; 2 – positive control, 3–9 skin biopsies from patients suffering from mycosis fungoides.

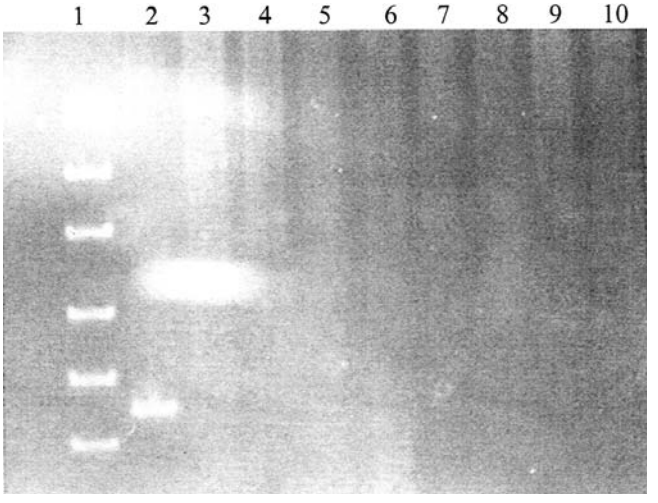

Figure 4. Nested PCR specific for tax region; 1 – molecular weight markers; 2 – positive control, 3–9 skin biopsies from patients suffering from mycosis fungoides.

specific for U5 and gag regions (primers: 1U5/2U5 and p19a/p19b, respectively) were designed. With these primer pairs no PCR product, except that in positive control, was observed (Fig. 2). The amplification was increased using nested-PCR with specific pol and tax primers, as reported in previous study by TOSSWILL et al [17]. PCR products were neither detected using these sets of primers (Fig. 3 and 4, respectively).

Discussion

Viruses from the Retroviridae family have been suspected for many years in the etiology of CTCL, however HTLV-I is a geographically restricted retrovirus associated with adult T-cell lymphoma, mainly confined to south-western Japan, the Caribbean Islands, south-eastern USA and Africa. Electron microscopy is used to visualize viral particles *in vitro* and *in vivo* [8, 20, 21] but morphologic data alone are not conclusive evidence for the presence of a retrovirus and must be confirmed by additional methods [9]. Molecular biologic studies have been performed in which the HTLV-I genome has been isolated from cutaneous lesions or cell lines from patients with MF [5, 7, 20, 21]. HTLV-I pol or tax proviral sequences in isolated peripheral blood mononuclear cells in 92% of patients with MF have been determined using PCR by ZUCKER-FRANKLIN and PANCAKE [21]. The presence of viral infection may also be investigated by the detection of antibodies to viral proteins in the patients' sera, however, the EIA test performed in our study gave negative results in all examined sera samples. The vast majority of patients with MF or SS fail to produce antibodies to HTLV-I/II [3, 13, 21]. Commercial kits contain only lysates of HTLV structural proteins. Therefore, if the virus is maintained in a replication-inactive form in which only the tax antigen is released, it is not possible to detect the antibodies to regulatory protein tax [21], although several authors have detected HTLV-I proviral sequences in high percentage of USA patients' sera [4, 6, 11, 20], European based studies have not confirmed these results [1, 2, 3].

Since the presence of HTLV antibodies or viral p15, U5, pol or tax sequences were not detected in any of our sera samples, we concluded that HTLV-I probably does not play

an important role in the pathogenesis of MF in Polish patients.

Approximately 1% of human genome consists of retroviral elements, including HRES-1 which contains HTLV-I LTR and gag-related sequences [12]. This may explain the discrepant results reported by different investigative groups. Recent study by SHOHAT et al [13] indicated that HTLV-I played a cofactor role in some of MF/SS patients.

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