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Absence of HTLV-Related Sequences in Skin Lesions and Peripheral Blood of Cutaneous T-Cell Lymphomas

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TO THE EDITOR

The involvement of retroviruses and more specifically of viruses belonging to the human T-cell lymphotropic virus (HTLV) family in cutaneous T-cell lymphomas' (CTCL) pathomechanisms remains a fiercely debated issue. Indeed, this hypothesis has remained attractive owing to the presence of a number of similarities between subsets of HTLV-1-associated adult T-cell leukemia and erythrodermic forms of CTCL. Overall, studies conducted in Europe have been generally negative (Bazarbachi *et al.*, 1993, 1997) with the notable exception of a report from Italy, which claimed the isolation of a new retrovirus distantly related to HTLV-1 (then designated HTLV-V) from a continuous cell line derived from a patient with CD4+ Tac− CTCL/leukemia as well as in other patients with CTCL (Manzari *et al.*, 1987). This alleged breakthrough has not been confirmed by subsequent studies, including further investigations conducted by the same team. Another, more recent, report identified HTLV-1 *tax*-like sequences in blood and saliva from Russian CTCL patients, and established that these sequences were indeed expressed up to the protein level (Morozov *et al.*, 2005). In addition, a significant proportion of USA-based investigations seemed to find out precise clues regarding the presence of retroviral agents close to HTLV-1 in skin lesions and/or peripheral blood in CTCL, and some authors have considered it reasonable to conclude that mycosis fungoides/Sézary syndrome was an HTLV-associated disease (Hall *et al.*, 1991; Pancake *et al.*, 1995; Khan *et al.*, 1996) even though other studies originating from the same geographical area have remained negative, as have been most of European studies (Wood *et al.*, 1997). The discrepancy in the results and concepts developed in these reports is puzzling, and the fact that these different studies have been conducted with heterogeneous tools makes it difficult to apply strict comparisons. Furthermore, virtually all tools used in these studies, of which most of them have been conducted more than 10 years ago, were either poorly specific (serological tests or identification of viral particles, for instance), or were instead specifically targeting HTLV-1 and notably the *tax* sequence, thereby significantly reducing the chances to uncover even slightly divergent retroviruses. To overcome this difficulty, we used a recently described powerful semi-nested DNA amplification method (Kim *et al.*, 2006), allowing the amplification of a sequence from the HTLV envelope gene located in the envelope receptor-binding domain (Kim *et al.*, 2004). This PCR amplification of the highly variable envelope region, as

Abbreviations: CTCL, cutaneous T-cell lymphoma; HTLV, human T-cell lymphotropic virus; PTLV, primate T-cell lymphotropic virus

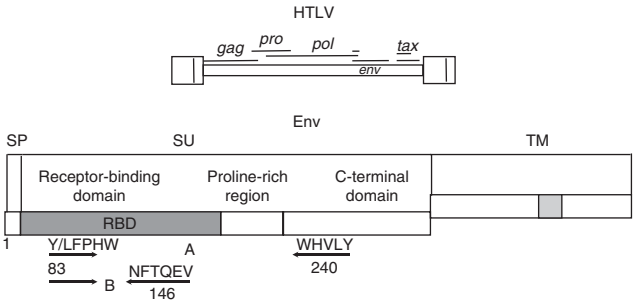

Figure 1. Schematic representation of the human T-cell lymphotropic virus (HTLV)-1 proviral genome, the encoded envelope glycoprotein, and the PCR amplification strategy. The *gag*, *pro*, *pol*, *env*, and *tax* genes are indicated with their respective open reading frames shown as bold lines on top of the provirus. Arrows indicate the localization of the primers across the envelope glycoprotein with the amino acids corresponding to the primer sequence shown on top, using the single letter amino acid code and numbering starting from the first signal peptide (SP) methionine of the HTLV-1 Env (envelope) precursor. A and B delineate the amplicons obtained after the first and second round of the semi-nested PCR protocol, respectively. SU, surface component of the envelope glycoprotein; and TM, transmembrane component of the envelope glycoprotein.

opposed to the conserved *tax* sequence, was designed to function with all known Primate T-cell lymphotropic viruses (PTLV) (Kim *et al.*, 2006). We applied this method to a wide search of PTLV sequences in a series of CTCL skin and blood samples.

Genomic DNA was extracted from the cutaneous lesions and from the peripheral blood mononuclear cells of 30 patients with CTCL diagnosed on usual clinical, histological, immunological, and molecular grounds (21 with mycosis fungoides stage Ib–IIb and nine with Sézary syndrome). Informed, written patients' consent and institutional approval for experiments were not required by French laws for the search of viral genetic material on tissue and blood samples obtained earlier for other purposes, and the experiments were conducted in accordance with Helsinki Guidelines. For amplification of HTLV-related sequences, up to 1 µg of genomic DNA was subjected to semi-nested envelope receptor-binding domain amplification under touchdown PCR conditions as described earlier (Kim *et al.*, 2006). A schematic representation of the strategy used is depicted in Figure 1.

Amplification of all 60 skin and blood samples from patients was all negative except for a single skin sample obtained from a patient with Sézary syndrome, wherein an amplimer of the apparent expected size (around 200 bp) was observed (not shown). However, subsequent sequencing of the amplified fragment displayed a non-related sequence, indicative of an isolated non-specific amplification. Conversely, positive controls, including HTLV-1 and 2, as well as STLV-3 sequences resulted in a successful amplification with the expected size and sequence of the amplimer, thus ensuring the validity and specificity of the results.

This study had been designed as an attempt to further assess the discrepancies between different studies with regard to the possible involvement of lymphotropic retroviruses in CTCL pathomechanisms. One hypothesis was that different viruses were present in different parts of the world and that tools specifically targeting HTLV-1 could not efficiently detect every possible PTLV variant. Accordingly, it became necessary to use a new, powerful, and highly sensitive tool that would ensure the detection of all known PTLV, including the recently described HTLV-4 (Switzer *et al.*, 2009). This study overall confirmed earlier generally negative reports with regard to an HTLV-related etiology for these syndromes. One hypothesis to explain the discrepancy in detecting HTLV in these diseases is hat initial, long-lasting stages of a particular, indolent (smoldering) subset of adult T-cell leukemia might have been mistaken with CTCL in studies issuing positive results with regard to HTLV-1-related virus search. However, in spite of limited similarities, most clinical and histological data are specific enough to prevent such confusion, especially in patients with protracted disease, and this possibility is thus very unlikely.

Eventually, our results do not definitively rule out the involvement of a PTLV in the oncogenesis of CTCL, as the intervention of a defective virus primarily or secondarily lacking the envelope gene remains theoretically possible (Hall *et al.*, 1991; Morozov *et al.*, 2005). Accordingly, studies must keep going in this still attractive perspective.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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