Characterization of a New Simian T-Lymphotropic Virus Type 1 (STLV-1) in a Wild Living Chimpanzee (*Pan troglodytes verus*) from Ivory Coast: Evidence of a New STLV-1 Group?

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ABSTRACT

A new strain of simian T-lymphotropic virus type 1 in blood samples from a chimpanzee that lived in the tropical rainforest of Ivory Coast is described. The sequence obtained from the long terminal repeat region of the genome is significantly divergent from all known human and nonhuman primate T-lymphotropic virus type 1 strains (96.3% homology to the closest related strains from Central African subtype B) and clusters with none of the established clades. The tax sequences reveal two sequence differences that seem to be unique as they are not found in any of the HTLV-1 or STLV-1 tax sequences from the public databases.

**STLV** and human T-lymphotropic virus type 1 (HTLV-1) are closely related. STLVs have been found in numerous nonhuman primate species.1–5 STLV-1 and HTLV-1 nucleotide sequence homologies range from 85 to 98%. However, some of the most closely related STLV-1 and HTLV-1 strains are less divergent than are HTLV-1 subtypes compared with each other.5 These observations suggest multiple interspecies transmissions, both among different primate species and between primates and humans.1,5–9

In this study we present sequence data for an STLV strain isolated from a chimpanzee (*Pan troglodytes verus*) living in its natural habitat, the tropical rainforest of Africa.10 The chimpanzee, called Leo, was the alpha male of a study group of free-ranging chimpanzees living under human observation since 1998 in the Tai National Park, Ivory Coast. Leo was about 19 years old when he died a sudden death on February 14, 2002. So far, the definitive reason for his death has remained unexplained. Full blood was taken during necropsy, placed immediately in aliquots in liquid nitrogen, and stored at −70°C until further use.

The blood sample was analyzed for HTLV-specific antibodies in an enzyme-linked immunosorbent assay (HTLV-1/2 ELISA; Murex Biotech, Dartford, UK). The positive ELISA result was confirmed by Western blotting (HTLV blot version 2.4; Genelabs Diagnostics, Singapore). The reaction profile was indistinguishable from the HTLV-1-positive control serum, with strong reactivities against p19, p24, gp46, rgp46-1, and GD21, suggesting that the chimpanzee was infected with an HTLV-1-related virus.

Aliquots of the frozen blood were thawed and DNA was extracted, using a QIAamp DNA blood minikit (Qiagen, Hilden, Germany). The absence of PCR inhibitors from the DNA sample was verified by a real-time PCR (TaqMan PCR) assay based on the sequence of human endogenous retrovirus R (HERV-R, data not shown).

The DNA sample was further analyzed for the presence of HTLV-like proviral DNA, using HTLV-1 tax- and pol-specific real-time PCR assays (details of the method are described elsewhere). For tax PCR primers SK43 and SK44, and for pol PCR primers SK110 and SK111,11 were combined with sequence-specific fluorescently labeled TaqMan probes. For each reaction 500 ng of total DNA was used. Each assay was run in duplicate, including appropriate positive and negative controls. Using standards with defined copy numbers of the target DNA, 50 proviral copies in the PCR assay was determined for both the pol and the tax region. The number of STLV-infected cells could be calculated as approximately 1 in 1000 cells.

In three independent PCRs starting from two independent DNA preparations, tax PCR product was generated with primers

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The 160-bp product was analyzed by agarose gel electrophoresis and the sequence was determined on both strands, using SK43 and SK44 as sequencing primers. The amplimers were sequenced with an ABI Prism BigDye terminator cycle sequencing Ready Reaction kit (PE Applied Biosystems, Oak Brook, IL).

The 118-bp tax sequence internal to the primers, which is usually well conserved among HTLV-1 and STLV-1 isolates, revealed two sequence differences compared with the HTLV-1 ATK prototype (accession number J02029): an A for a G at position 7433 and a T for a C at position 7482. The two substitutions seemed to be unique as they were not present in any of the published HTLV-1 or STLV-1 tax sequences. To further characterize this isolate, the 3' region of the STLV genome was sequenced and investigated by phylogenetic tree analysis.

To amplify tax and the long terminal repeat (LTR) region, we used primer sequences derived from HTLV-1 ATK. Primers SK43 and LTR R2 were used in the first PCR to amplify a 1.6-kb fragment covering tax and rex as well as the 3' LTR (nucleotide positions 7357 to 9036 in ATK). Because in agarose gel electrophoresis no clear PCR fragment of the expected size could be identified after first-round PCR, primers

![Phylogenetic Tree](image)

**FIG. 1.** A rooted phylogenetic tree, generated by the neighbor-joining method and based on a 665-bp LTR fragment corresponding to nucleotide positions 8331 to 8995 of the ATK sequence. HTLV-1 subtype isolate Mel 5 was used as an outgroup. The tree was statistically evaluated in a bootstrap analysis with 1000 bootstraps. Bootstrap values for the major branching points are given as percent values. The different HTLV-1 subtypes are indicated. The Ptr-Leo isolate is highlighted in a shaded box.
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SEQUENCE DATA

Accession numbers: agm-29752 (AF 061847), ALT-YS (U19949), ANG-MER (AY026858.1), ANG-VEN (AY026857.1), ANG-WIS (AY026856.1), ATK1 (J00209), CAE-AGM511 (AY026847.1), CAE-AGM535 (AY026846.1), CAE-89032 (AY026851.1), CAE-97022 (AY026850.1), CAE-AGM03 (AY026848.1), CAE-1301 (AY026852.1), CAG-DJA-853 (AF384872), CHPSSTLVPRO (M33064), CMI-MIT (AY026845), CMO-1401 (AY026853.1), CNI-1001 (AY026851.1), CTO-601 (AY026855.1), Ef1 (Y17014.1), GAB7 (L76311.1), Gb233 (D23692), H23 (L76312), H24 (L76308), Lib1 (Y17016.1), Lib2 (Y17017), Lib3 (Y17018.1), MEL5 (L02534), mnd9 (AF045932), mnd13 (AF045933), MSP-SAN-855 (AF384870), MT2 (L03561), OD (U12805), PAN-614 (AY026844.1), PAN-622 (AY026843.1), PAN-623 (AY026842.1), PH236 (L76307.1), Pr126 (U86376), Pr-TAR-875 (AF384871.1), Pyg19 (L76310), RK11-Iran (AF030310), RK13-Ger (AF042071.1), StDen (L76306.1), TBH2 (L76025), T49 (L76305), 12503 (L76309.1).

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