Characterization of a simian T-lymphotropic virus from a wild-caught orang-utan (Pongo pygmaeus) from Kalimantan, Indonesia

Ernst J. Verschoor,1 Kristin S. Warren,2, 3 Henk Niphuis,1 Heriyanto2, 4, Ralph A. Swan3 and Jonathan L. Heeney1

1 Department of Virology, Biomedical Primate Research Centre (BPRC), Lange Kleiweg 157, 2288 GJ Rijswijk, The Netherlands
2 Wanariset Orang-utan Reintroduction Centre, Samboja, East Kalimantan, Indonesia
3 Division of Veterinary and Biomedical Sciences, Murdoch University, Perth, Australia
4 Department of Quarantine, East Kalimantan, Indonesia

In a recent serological survey among 143 ex-captive orang-utans two individuals were found that reacted positive in an ELISA detecting antibodies which cross-react with human T-lymphotropic virus type I (HTLV-I) antigens. Infection of both animals with an HTLV-I or simian T-lymphotropic virus (STLV)-like virus was confirmed by Western blot analysis. A third wild-caught animal, which was not part of the original serological survey, was also found to be infected with an HTLV-related virus in a diagnostic PCR assay and Western blot assay. Nucleotide sequence analysis of the 709 bp PCR fragment from the tax/rex region of the HTLV/STLV genome confirmed infection of orang-utans with an STLV similar to but clearly distinct from other Asian STLVs.

Human T-cell lymphotropic virus types I (HTLV-I) and II (HTLV-II) are retroviruses that are aetiologically associated with adult T-cell leukaemia/lymphoma (ATL) and tropical spastic paraparesis (TSP) (HTLV-I) and rare, isolated cases of hairy cell leukaemia (HTLV-II). Viruses closely related to HTLV-I and -II have been characterized from a large number of African, Asian and New World primate species. These viruses, called simian T-cell lymphotropic viruses or STLVs have not been linked to any specific disease in their natural hosts. Phylogenetic analyses of STLV and HTLV isolates have suggested that HTLV-II is genetically distinct and separated from a common ancestor virus before HTLV-I evolved from

Author for correspondence: Ernst J. Verschoor.
Fax +31 15 284 3986. e-mail verschoor@bprc.nl

The EMBL Nucleotide Sequence Database accession number for STLV_OU_KA is Y13146.

Fig. 1. Western blots of sera of orang-utans (Pongo pygmaeus) positive in HTLV-ELISA (OU-BA, lane 3; OU-CC, lane 6) or tax/rex PCR (OU-KA, lane 10). Sera of individuals negative in the HTLV-I ELISA were OU-BA, lane 4; OU-CL, lane 5; OU-IU, lane 7; OU-SI, lane 8 and OU-MU, lane 9. Lanes 1 and 2, blots incubated with positive and negative control sera, respectively. Analysis of sera was performed according to the manufacturer’s instructions, except that for OU-KA a 1:100 dilution of a preserved EDTA-treated blood sample was used as the sole available sample.
Fig. 2. Alignment and comparison of the (A) p40\textsuperscript{tax}, (B) p27\textsuperscript{rex} and (C) p13/p30\textsuperscript{rex} amino acid sequences encoded by the 709 bp PCR fragment of STLVOU-KA with those of HTLV-IATK, HTLV-IMEL-5, STLVPTM3 and STLVTE4. Only differences with the STLVOU-KA sequence are shown. * indicates a stop codon.

STLV during multiple interspecies transmissions (Koralnik \textit{et al.}, 1994; Saksena \textit{et al.}, 1994; Song \textit{et al.}, 1994). Also, there are indications for an Asian origin of the HTLV/STLV viruses (Song \textit{et al.}, 1994; Giri \textit{et al.}, 1997). For the great apes, chimpanzee, gorilla and orang-utan, STLVs have been characterized from the common chimpanzee (\textit{Pan troglodytes}) (Koralnik \textit{et al.}, 1994) and the pygmy chimpanzee (\textit{P. paniscus}) (Giri \textit{et al.}, 1994; Vandamme \textit{et al.}, 1996), while antibodies cross-reacting with STLV have been described in gorillas (\textit{Gorilla gorilla}) (Ibrahim \textit{et al.}, 1995). Here we describe the characterization and phylogenetic relationship of an STLV isolated from the only great ape species found outside of Africa, the orang-utan (\textit{Pongo pygmaeus}).

As a part of a serological survey among 143 ex-captive orang-utans at the Wanariset Orang-utan Reintroduction Centre, Kalimantan, Indonesia, two animals, ChiChi (OU-CC) and Belle (OU-BE), were found to have antibodies that cross-reacted with HTLV antigens by ELISA (K. Warren, H. Niphuis, Heriyanto, E. Verschoor, R. Swan & J. L. Heeney, unpublished results). Confirmation of the infection with a HTLV-like virus was obtained using a commercial HTLV Western blot (WB) (HTLV Blot 2.4, Genelabs Diagnostics). In Fig. 1, lanes 3
sequences shows 89% identity with the prototype HTLV-I isolate ATK from Japan (Cosmopolitan HTLV-I subtype), 90% with HTLV-I\textsubscript{Mel}, from the Solomon Islands (Melanesian subtype), 92% with STLV\textsubscript{PTM}, isolated from Macaca nemestrina from Indonesia and 93% with STLV\textsubscript{TEI} from Macaca tonkeana, also from Indonesia. The amino acid sequence similarities for the 236 amino acid p40\textsuperscript{tax} sequence were 92, 94, 92 and 93%, for the p27\textsuperscript{rex} 181 amino acid peptide 86, 85, 84 and 85%, while the identities for the 65 amino acids of p13/p30\textsuperscript{rex} were 92, 93, 81 and 93%, respectively. The high similarity between strains from different HTLV-I clades (ATK and MEL5) and from STLV strains originating from the same geographical region but isolated from other species is not surprising as tax/rex is the most conserved region among STLVs, HTLV-I and HTLV-II (Sherman et al., 1992; Shaw et al., 1984). The strong conservation of the tax/rex region makes it particularly suitable for determination of phylogenetic relationships between HTLVs and STLVs (Giri et al., 1997). The short 118 bp tax/rex fragment from the diagnostic PCR was used to construct a phylogenetic tree using the neighbour-joining method as implemented in the MEGA package (Kumar et al., 1993) (Fig. 3). Clearly STLV\textsubscript{OU-KA} falls within the HTLV-I/STLV-I evolutionary cluster as described by Giri et al. (1997) but constitutes an isolated member of the subcluster consisting of Asian STLV-I strains from India, Japan and Indonesia.

We have characterized an STLV from a naturally infected orangutan. Sequence analysis of a fragment from the tax/rex region of the genome showed a 7–10% nucleotide difference with other, geographically related HTLV-I and STLV viruses, while their amino acid sequences were 6–19% divergent from that of STLV\textsubscript{OU-KA}. Distinct amino acid differences in the sequence of STLV\textsubscript{OU-KA} indicate that we have indeed characterized a new STLV from orang-utans. Asian STLV and Melanesian HTLV-I viruses tend to be more divergent in their tax/rex genes (8.5% difference in nucleotide sequence between HTLV-I\textsubscript{ATK} and HTLV-I\textsubscript{Mel}) than African strains (97–99% similarity) (Gessain et al., 1993). Based on this, STLV\textsubscript{OU-KA} clusters in a recently proposed new clade consisting of Melanesian HTLV-I subtype C and Asian STLVs (Mahieux et al., 1997). Thus, STLV\textsubscript{OU-KA} is not closely related to the STLVs found in the other great apes (STLV-I ChM114.1, STLV-II PP166 and STLV-II L93; see Fig. 3), albeit that these apes are closely related to orang-utans, but rather to regional viruses, supporting the concept that they were obtained long after speciation and geographical separation.

Typing of the orang-utan STLV using WB remained inconclusive as no reaction was detectable with the HTLV-I and HTLV-II marker proteins. Comparable serological profiles, i.e. a lack of reactivity with HTLV-I and -II envelope antigens, were found when sera from STLV-infected pygmy chimpanzees (P. paniscus) were analysed on WB (Giri et al., 1994; Vandamme et al., 1996; Digilio et al., 1997). Recently, this virus (STLV\textsubscript{pan}p) was reported to be more related to HTLV-II and STLV-II from New World monkeys (Digilio et al., 1997).
1997). Although STLV_pan-p is clearly more divergent in the tax/rex region than STLV_OU-KA (79.7, 78.9 and 78.8% similarity with HTLV-I_ATK, HTLV-I_MEL and STLV_PTMA, respectively) (Giri et al., 1994), it is tempting to speculate on the divergence of the envelope gene of STLV_OU-KA compared to that of other Asian/Melanesian isolates. Attempts to amplify envelope gene fragments of STLV_OU-KA using a semi-nested PCR with primers env-1, env-2 and env-22 (Gessain et al., 1992) and a nested PCR (Liska et al., 1997) were unsuccessful despite the proven sensitivity of the assays for a broad range of HTLV-I and STLV-I viruses (E. Verschoor, unpublished results). This would suggest a greater divergence in the envelope region of the STLV_OU-KA genome compared to other HTLV-I and STLV isolates implying a unique phylogenetic niche between the HTLV-I and HTLV-II viruses. Further analysis using degenerate PCR primers to conserved sequences among HTLV-I and HTLV-II may give more insight into the evolutionary relationship of STLV_OU-KA and the other T-lymphotropic viruses.

The authors wish to thank Dr A. van der Kuyl for her help in performing phylogenetic analyses. We thank the staff at the Wanariset Orangutan Reintroduction Project, in particular Ir. A. Susilo, Dr W. Smits, Amanudin and F. Hartono, and LIPI (Indonesian Institute for Scientific Research) for enabling research at the centre. Work of K.S.W. was financially supported by PT Kaltim Prima Coal, CRA Foundation, PT Kelian Equatorial Mining and the Merck Foundation.

References


Received 30 June 1997; Accepted 11 September 1997