The presence of a divergent T-lymphotropic virus in a wild-caught pygmy chimpanzee (Pan paniscus) supports an African origin for the human T-lymphotropic/simian T-lymphotropic group of viruses

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We isolated a divergent simian T-lymphotropic virus (STLV) (strain PP1664) from a wild-caught African bonobo (pygmy chimpanzee, Pan paniscus). Molecular and phylogenetic characterization of this virus show that it reliably separates from the two well-established primate T-lymphotropic virus types, HTLV-I/STLV-I (PTLV-I) and PTLV-II, and from a third type isolated from an African-born Papio hamadryas and designated by us as PTLV-L. Four of eight bonobos kept at the Antwerp Zoo, Belgium, showed an aberrant PTLV serology. We amplified and sequenced a 709 bp PTLV proviral tax/rex fragment from one of the reactive bonobos. It differs by about 25% from the homologous nucleotide sequences of PTLV-I and PTLV-L and by about 17% from PTLV-II. This is comparable to the differences among the three known types. Including the most divergent STLV-I strains sequenced to date, for example, strain PHSul sequenced here, the divergence in this region within PTLV-I is less than 11% and within PTLV-II less than 4%. Although very divergent, this new bonobo STLV is the closest well-characterized simian relative of HTLV-II, raising the possibility of very divergent new HTLV strains. Our results show that the number of PTLV types should be considered open and that the variety of indigenous viruses in the PTLV group is greatest in Africa. Thus, as for the other primate retroviruses HIV and SIV, PTLV most probably has its origins in Africa.

Introduction

Human T-lymphotropic virus types I and II (HTLV-I and -II) are retroviruses associated with neurological degeneration and leukaemia in a small number of infected individuals. Since HTLV and simian T-lymphotropic virus (STLV) cannot be separated into distinct phylogenetic lineages according to species of origin, several authors have used the term primate T-lymphotropic viruses (PTLV) (Goubau et al., 1994; Saksena et al., 1994; Vandamme et al., 1994; Watanabe et al., 1986). The very stable genome of these viruses provides an excellent tool for investigating the origin of PTLV in general and of HTLV-I and HTLV-II in particular (Gessain et al., 1992; Komurian et al., 1991; Liu et al., 1994a). The discovery of divergent Asian STLV-I (Song et al., 1994; Watanabe et al., 1986) and Asian and Australian HTLV-I strains (Bastian et al., 1992; Gessain et al., 1993) together with the presence of HTLV-II among native Amerindians (Ferrer et al., 1993; Lairmore et al., 1990; Maloney et al., 1992) support an Asian origin of PTLV. However, recent phylogenetic studies using divergent African and Asian STLV-I strains support an African origin of the African and cosmopolitan HTLV-I strains, and an Asian origin of the Melanesian and Australian HTLV-I strains, implying several separate interspecies transmissions (Koralnik et al., 1994; Saksena et al., 1994; Song et al., 1994; Vandamme et al., 1994). The available data do not allow the origin of PTLV-I to be traced to Asia or to Africa. The American origin of HTLV-II is now also being questioned, due to the discovery of HTLV-II in indigenous African pygmy tribes (Froment et al., 1993; Goubau et al., 1992, 1993, 1996; Gessain et al., 1995) and a distinct molecular HTLV-IIb variant in Gabon.
(Gessain et al., 1994). We recently discovered a new type of STLV in a wild-caught African baboon (Gobau et al., 1994), which we provisionally called PTLV-I, since HTLV-III and HTLV-IV/STLV-IV have previously been used for the immunodeficiency viruses HIV-1 and HIV-2/SIV respectively. This PTLV-I is as different from PTLV-I and PTLV-II as both are from each other.

We recently reported the presence of yet another divergent STLV (PP1664) in a wild-caught African bonobo (pygmy chimpanzee, Pan paniscus) (Liu et al., 1994). We now describe the isolation, and the phylogenetic and partial genetic characterization of this new STLV and discuss the implications of the data for the origin of PTLV. Additionally, sequencing the tax/rex region of a very divergent STLV-I strain (PHsul), derived from a captive-born baboon from the Sukhumi primate centre, and of three Zairian HTLV-I strains allowed us to extend present phylogenetic analyses of PTLV-I in this region. Together these data provide further evidence for an African origin for PTLVs and clearly show that the present classification of PTLVs into two types, PTLV-I and PTLV-II, should be extended. The discovery of these new simian retroviruses encourages the search for related human viruses.

Methods

Immunological assays. Sera from eight bonobos (pygmy chimpanzees, Pan paniscus) collected in the Antwerp Zoo were screened for HTLV-I or -II cross-reacting antibodies with a particle-agglutination assay (Serodia-HTLV, Fujirebio, Tokyo). All sera were also tested with a modified HTLV-I immunoblot assay spotted with recombinant transmembrane protein (g3321) cross-reacting with both HTLV-I and -II, and with the recombinant proteins MAT-1 and K55, derived from the outer membrane protein, and specifically recognized by HTLV-I and HTLV-II antibodies, respectively (Genelabs, Singapore). The sera were also tested with type-specific ELISAs that use synthetic peptides derived from the matrix protein of HTLV-I and from the outer membrane protein of HTLV-II (Select HTLV, IAF-Biochem, Montreal, Canada). Additionally, an indirect immunofluorescence assay on MT-2 cells (HTLV-I producing), on clone 19 cells (HTLV-II producing; Gallo et al., 1991) and on PH969 cells (PTLV-L producing) (Gobau et al., 1994) was performed.

Origins of the PTLV strains studied here. All new sequences presented here are derived from stable cell lines harbouring HTLV or STLV proviral sequences. The establishment of the continuous HTLV-I (ITIS, MOMS and MWMG, all three from Zaire) or STLV-I (PHsul from the Sukhumi primate centre) producing cell lines has been described previously (Vandamme et al., 1994). Similarly, a continuous STLV bearing lymphoid cell line was obtained from one of the reactive bonobos by coculture of its lymphocytes with human cord blood lymphocytes. The production of P24 core antigen in the culture fluid was monitored with a capture ELISA that detects HTLV-I, HTLV-II and PTLV-I (Coulter). This STLV PP1664 cell line was derived from lymphocytes of a wild-caught female bonobo (Zaire). The bonobo was transported to Antwerp in 1992, after residing at the Institute and Museum of Primatology, Innyani, Aichi, Japan. Sera XZ181 to XZ187 (see Fig. 1) were all derived from wild-born (Zaire) bonobos with no familial relationship to PP1664, except for the seronegative XZ181 that was born in Antwerp. The origins of the other isolates used in the phylogenetic analysis are given in Table 1.

PCR and sequencing. For provirus detection in the cell lines, 10^6 cells were pelleted and lysed in PCR buffer (10 mM-Tris–HCl pH 8.3, 50 mM-KCl; Perkin-Elmer) containing 2 mM-MgCl_2, 0.5% Tween 20, 0.5% NP40 and 100 μg/ml protease K (Boehringer Mannheim, stabilized protease K solution) for 1 h at 56 °C. The DNA was extracted using phenol–chloroform (Life Technologies), precipitated with ethanol and dissolved in Milli-Q water (Millipore). DNA from 10^6 cells was used for amplification with the nested TR101–104 primer set (tax region; Maloney et al., 1992) or with the nested PCR primer set TR101–HFL102 (outer)/TR103–HFL104 (inner) (HFL102, 5' TGI(A/T)A(G/C)TAC(I/C)TAAAGATGGCCTG 3' (antisense, complement to nt 8115–8134 of HTLV-I ATK1); HFL104, 5' GG(I/C)T(A/G)GGICCGGAAATCAT 3' (antisense, complement to nt 8085–8103 of ATK1)). All amplifications were done in PCR buffer, 200 μM nucleotide triphosphates, 0.8 μM outer primers or 0.5 μM inner primers and 0.025 U/μl AmpliTag (Perkin-Elmer) using 3 mM-MgCl_2 for the TR primers and 2 mM-MgCl_2 for the TR/HFL primer sets, in a 50 μl reaction volume. The PCR was done with a GeneAmp PCR system 9600 (Perkin-Elmer) under the following conditions: 30 s at 95 °C, 30 s at 50 °C, 45 s at 72 °C. The outer primer was amplified for 35 cycles, 2 μl was transferred to the inner PCR and amplified for 25 (TR primer set) or 35 (TR/HFL primer set) cycles. Amplification products were separated on a 6% polyacrylamide gel and visualized by ethidium bromide staining.

After purification of the PCR product on a 1.3–3% agarose gel using a Sephaglass Bandprep kit (Pharmacia), 50 fmol was used for sequencing with the dsDNA Cycle Sequencing System (Life Technologies). The sequencing primers were TR102, TR103, HFL101 (5' ACCTGCGGGAACCGTTA 3'), HFL103 (5' CCACCGAGAGA CCTATA 3'), HFL104, HFL105 (5' CTCC(T/C)TG(T/C)CGCC(T/C)CTATG TATTG 3'), HFL106 (5' GGGATAGTGTAGGAGTTA 3') and HFL108 (5' GGCA(G/A)GAC(A/G)GTGTGGAGGA 3'). Sequencing fragments were separated on a Sequi-Gen Cell sequencing unit (Biorad).

The oligonucleotides were synthesized on a Gene Assembler Plus DNA synthesizer by Pharmacia Biosystems or on an Applied Biosystems DNA synthesizer model 381A by A. van Aerschot (Rega Institute, Leuven, Belgium).

Sequence analysis and phylogeny construction. Sequence alignments were performed using the GeneWorks software package (IntelliGenetics). For the alignment of bovine leukaemia virus (BLV) (Sagata et al., 1985) with PTLV, a pairwise nucleic acid and protein (PAM250 score) dot matrix comparison (implemented in GeneWorks) was performed on BLV with HTLV-I ATK1, HTLV-II Mo, PTLV-L and the new STLV PP1664. The Tax protein similarity was used as an indication for the nucleotide alignment. Phylogeny construction and evaluation were done using the Phylib software package (Felsenstein, 1989). A neighbour-joining (NJ) tree was constructed using Kimura 2-parameter distances, where transitions were scored twice more likely than transversions. A nucleotide state changes chart was made from the alignment using the MacClade (version 3) software package (Sinuva Associates). This chart was converted into a cost matrix using the function $K_{ij} = -\ln(X_{ij}/X_i)$ where $K_{ij}$ is the cost of going from state $i$ to state $j$, $X_{ij}$ the number of $i$ to $j$ changes and $X_i$ the number of changes from $i$ to any state, where $i$ and $j$ are nucleotide character states. The cost matrix was corrected according to the triangular rule. For the analysed alignment, the cost matrix can be described as symmetrical, with a transition/transversion bias of about seven. Next, three different methods implemented in the Phylib package were used: the NJ method, the Fitch and Wagner parsimony (pars) method and the maximum likelihood (ML) method. For all methods, transitions were scored
Table 1. Origins of the PTLV isolates used in this study

<table>
<thead>
<tr>
<th>Type</th>
<th>Strain</th>
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<th>Geographical origin</th>
<th>Reference</th>
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<td>USA</td>
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<tr>
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<td>PH969</td>
<td>Papio hamadryas</td>
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<td>Van Brussel et al. (1996)</td>
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<td>Zaire (Antwerp Zoo)</td>
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<td>Z46344</td>
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</table>

seven times more likely than transversions. Distances were calculated with the Felsenstein model which uses the empirical base frequencies. The NJ and pars trees were statistically evaluated using 1000 bootstrap samples (Felsenstein, 1985). No bootstrapping was done for the ML method, which is itself already a statistical method. The values on the branches represent the percentage of trees for which the sequences at one end of the branch are a monophyletic group.

Results
Isolation of an STLV from a bonobo with an aberrant serological profile

Using the screening assay Serodia-HTLV, four of eight sera collected in the Antwerp Zoo from unrelated wild-born bonobos tested HTLV positive (XZ007, XZ184, XZ185 and XZ186; see also Fig. 1). In a Western immunoblot, the positives reacted with both Gag proteins p24 and p19, with the recombinant gp21 transmembrane Env protein and with the HTLV-I-specific MTA-1 protein (Fig. 1). Reactivity with the recombinant MTA-1 protein was to some extent also seen in the screening negatives. None of the sera reacted with type-specific HTLV-I and HTLV-II synthetic peptides in an ELISA. By immunofluorescence, the sera of three of the four seropositive bonobos reacted at high titre with the HTLV-II clone 19 cells and weakly with the HTLV-I MT-2 cells and the STLV PH969 cells, while the fourth had a lower titre against clone 19 cells and no cross-reactivity with MT2 and PH969 cells. From this fourth reactive bonobo (serum sample XZ007), peripheral blood mononuclear cells were available and a stable interleukin-2 dependent antigen producing cell line, PP1664, was obtained after cocultivation with phytohaemagglutinin stimulated human cord lymphocytes.
HTLV-II positive sera from pygmy people in Zaire and Cameroon only showed weak or no immunofluorescence reactivity towards the PP1664 cell line. The typical HTLV-II serological profile detected in these pygmies (Goubau et al., 1992, 1993) was also different from the aberrant profiles found in the infected Pan paniscus.

**Amplification and sequencing of a tax/rex fragment from the proviral genome of STLV PP1664, STLV-I PHSul and the Zairian HTLV-I strains**

From both the original lymphocytes and the PP1664 cell line, a 159 bp fragment was obtained in a nested PCR using the TR101–104 primers (Liu et al., 1994b; Maloney et al., 1992). The SK110–111 primers (Kwok et al., 1988) in the pol region gave negative results (data not shown). Sequencing of the 120 nt fragment (excluding the primers) revealed that this proviral tax/rex DNA fragment was from a very divergent STLV. We developed a nested primer set, TR101–HFL102/TR103–HFL104, conserved among PTLV-I, -II and -L. A 746 bp PTLV-like proviral tax/rex fragment was obtained from the stable cell line PP1664, from a cell line derived from a baboon at the Sukhumi primate centre in Georgia that harboured STLV-I PHSul, and from the Zairian HTLV-I cell lines (Fig. 2). Using the PCR and internal primers, we sequenced the 709 nt tax/rex fragment (excluding the primers) in both directions and compared them with prototype and divergent PTLV strains. The Zairian HTLV-I strains ITIS, MWMG and MOMS were all very similar to the reported Zairian EL strain. The STLV-I strain PHSul was the most divergent PTLV-I reported in this region, differing by about 11 % from all other PTLV-I strains (Table 2). STLV PP1664 differed by 26%, 17% and 27% from HTLV-I (ATK1), HTLV-II (Mo) and STLV-PH969 respectively. In this 709 nt region, PTLV-I strains differ by less than 11% and PTLV-II strains by less than 4%. At the amino acid (aa) level this partial Tax sequence is well conserved with 8–22% difference.
A new STLV from a pygmy chimpanzee

Fig. 2. Genomic organization of PTLVs. Narrow boxes represent ORFs. Tax and Rex are expressed from a double spliced mRNA using two overlapping ORFs. The 709 nt fragment sequenced and described in this paper is indicated.

Table 2. Comparison of the divergence between PTLV strains using the 709 nt tax/rex fragment

<table>
<thead>
<tr>
<th>Percentage nucleotide difference</th>
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<td>HTLV-Ib ITIS</td>
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<tr>
<td>HTLV-Ia ATK1</td>
</tr>
<tr>
<td>HTLV-Ib ITIS</td>
</tr>
<tr>
<td>HTLV-Ic Mel5</td>
</tr>
<tr>
<td>STLV-I PM3</td>
</tr>
<tr>
<td>STLV-I PHSul</td>
</tr>
<tr>
<td>HTLV-IIa Mo</td>
</tr>
<tr>
<td>HTLV-IIb Mo</td>
</tr>
<tr>
<td>NRA</td>
</tr>
<tr>
<td>PTLV-L PH969</td>
</tr>
</tbody>
</table>

Table 3. Comparison of the Tax and Rex amino acid divergence between PTLV strains

For details of the strains, see Table 1.

<table>
<thead>
<tr>
<th>Percentage amino acid difference (Tax)</th>
<th>Percentage amino acid difference (Rex)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>HTLV-Ia Mo</td>
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<tr>
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<td>HTLV-Ia Mo</td>
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<tr>
<td>PTLV-L PH969</td>
<td>12</td>
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<td>44</td>
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</table>

from the prototype PTLV strains (Table 3). The corresponding differences for this partial Rex fragment are substantially higher (30–47 %, Table 3). Giri et al. (1994) recently confirmed the existence of a very divergent STLV in pygmy chimpanzees. In the 120 nt fragment sequenced, their STLV PanP isolate differs by 3-3 % from STLV PP1664. A search through EMBL and GenBank did not show similar sequences outside the known HTLV and STLV sequences. No significant similarity was seen with the BLV tax/rex region. At the protein level, BLV Rex shows no similarity, but in a protein dot matrix the N-terminal part of BLV Tax shows a low similarity with the different PTLV types (Fig. 3).

Phylogenetic analysis of PTLV using the 710 nt tax/rex fragment

The complete 709 nt sequence, determined for STLV PP1664, was available in the literature and/or the EMBL/GenBank database for 25 strains. We performed an unrooted phylogenetic analysis on the corresponding 710 nt consensus sequence of the 23 different tax/rex
sequences (Table 1) among these 25 strains. In all three methods used, the main clusters were PTLV-I, PTLV-L, PTLV-II and PTLV-PP (STLV PP1664). The unrooted phylogenetic tree obtained with the NJ method is shown in Fig. 4. The bootstrap values that supported the separation of these four types were 100 % for the NJ and pars methods. This separation was also highly significant with the ML method (P < 0.01). The topology within PTLV-I was similar for the three methods used, NJ and ML being almost identical. There was a clear separation between all Zairian HTLV-Ib strains, all cosmopolitan HTLV-la strains and the Melanesian HTLV-Ic strain Mel5, the most divergent HTLV-I in this analysis. High bootstrap values support these three HTLV-I clusters in all methods used (Fig. 4). Only two STLV-I strains were available for this analysis: the Asian PtM3 and the Sukhumi PHSu1: both branched off before the HTLV-I Mel5. The separation from Mel5 was supported by only about 70 % of the bootstrap replicates (Fig. 4). The origin of the Sukhumi strain is obscure. The baboons in this colony originate from Africa, but there was close contact with Asian monkeys. According to the LTR (Vandamme et al., 1994) and the present tax analysis, this Sukhumi strain has an Asian-like sequence. No divergent African STLV-I strains were available for this analysis. Within PTLV-II, all methods support a highly significant separation between HTLV-IIa and HTLV-IIb (Fig. 4).

To be able to include STLV PanP in a phylogenetic analysis, the reported 120 nt tax/rex fragment was analysed. In all three methods used, the main clusters were again PTLV-I, PTLV-I, PTLV-II and PTLV-PP (STLV PP1664 and STLV PanP) with bootstrap values of 80–100 % (not shown). Both strains isolated from Pan paniscus cluster together in more than 97 % of the bootstrap replicates in NJ and pars, and with P < 0.01 in the ML method (implemented on Fig. 4). No clustering was observed according to the well established Asian, African or cosmopolitan clades for PTLV-I, or to subtype IIa or IIb for HTLV-II. Within both types, the topology was different with the different methods.

**Rooting the PTLV tree with BLV**

In a DNA dot matrix of the tax/rex gene, BLV did not show any similarity with other PTLV strains (not shown). At the protein level, a weak similarity was seen only in the N-terminal part of Tax (indicated on Fig. 3). The corresponding genomic fragment, spanning nt 1–168 of the sequenced STLV PP1664 tax/rex, was used to root the phylogenetic tree in Fig. 4. With all three methods, the BLV root node was located between PTLV-I and PTLV-L. The bootstrap values for the separation from PTLV-I (56.5 % in NJ, 93.3 % in pars, P > 0.05 in ML) and from PTLV-L, PTLV-PP and PTLV-II (> 80 % in NJ and pars, P < 0.01 in ML) were not very high. The BLV root node was on the STLV-I PHSu1 branch in 40.1 % of NJ and 5 % of pars replicates and on the PTLV-L branch in 31 % of NJ and 48 % of pars replicates. This makes the Sukhumi STLV-I PHSu1 the PTLV strain that is closest to BLV for this 173 nt consensus fragment.

**Discussion**

The primate T-lymphotropic viruses have so far been classified into two distinct groups, PTLV-I and PTLV-II. The very stable genomic information within and the genetic divergence between the groups suggests an ancient separation. This classification has been widened due to the recent discovery of a new STLV (PTLV-L) from a baboon (Papio hamadryas), genetically as distant from both groups as PTLV-I and PTLV-II are from each other (Goubau et al., 1994). We have identified another new very divergent STLV, isolated from bonobos (pygmy chimpanzees, Pan paniscus), with an aberrant HTLV-I and HTLV-II serological profile. The four infected bonobos showed HTLV-I reactivity in a Western blot, but HTLV-II reactivity in an immunofluorescence assay.
A new STLV from a pygmy chimpanzee

On a type-specific ELISA using synthetic peptides of HTLV-I and HTLV-II (Select HTLV), the sera were negative. Additionally, three Zairian HTLV-I strains and one STLV-I strain from the Sukhumi primate centre were characterized. These four strains showed a typical HTLV-I serological profile.

Goubau et al. (1992, 1993, 1996) have shown the presence in Zaire and in Cameroon of an indigenous African reservoir of HTLV-II in isolated pygmy tribes. The pygmy sera showed a typical HTLV-II profile, including HTLV-II reactivity on the type-specific ELISA Select HTLV. These findings suggest that this African Pan paniscus virus is not closely related to the African pygmy virus.

Giri et al. (1994) also described an STLV in pygmy chimpanzees with an aberrant serological profile. In the 120 nt fragment they reported without a phylogenetic analysis, their pygmy chimpanzee virus differed by 3.3% from STLV PP1664. In this short stretch of nucleotides these two new viruses differ by about 20% from PTLV-I but by only 11% from HTLV-II Mo with identical Tax amino acid sequences for STLV PP1664 and HTLV-II Mo. The nucleotide difference within HTLV-II is only 1%, but within PTLV-I it is up to 6%. Our present phylogenetic analysis of this 120 nt stretch showed that within PTLV-I or PTLV-II no clusters are apparent; all methods give different topologies and none are statistically supported. This very conserved sequence fragment is therefore not suitable for tracing the origin of each type separately. More sequence information for the new pygmy chimpanzee viruses is needed to judge with confidence whether these two STLVs represent a new type of PTLV or if they can be classified as a very divergent STLV-II.
Using the sequence information for PTLV-I, PTLV-II and PTLV-L, we have amplified the tax/rex genes of the new STLV PP1664 and four PTLV-I strains. The 709 nt fragment sequenced includes most of the second coding exon of Tax. It starts 50 nt downstream of the presumed tax/rex splice acceptor, includes the end of the Rex coding region and extends to about 240 nt before the presumed end of the Tax coding region. From the sequence comparison in this region it is now clear that this STLV PP1664 does not belong to the established PTLV-II than to the other types of PTLV. It is also very different from the newly discovered PTLV-L type (Goubau et al., 1994). In this fragment, the percentage difference between the three known PTLV types is about 25%. STLV PP1664 differs by the same amount from PTLV-I and -L, but by only 17% from PTLV-II. The known divergence within PTLV-I in this tax/rex fragment is less than 11%, and within PTLV-II it is less than 4%.

We performed a phylogenetic analysis to clarify whether STLV PP1664 represents a new type of PTLV. In the very stable region examined, all PTLV strains could be aligned unambiguously. The complete sequence homologous to the known 709 nt STLV PP1664 fragment is available for 23 different strains, resulting in a 710 nt consensus fragment. In all phylogenetic analyses, four major clades consistently appear (Fig. 4): PTLV-I, PTLV-II, PTLV-L and a clade including STLV PP1664 and STLV PanP which we call PTLV-PP. The separation between PTLV-II and PTLV-PP using the 120 nt consensus fragment (to be able to include STLV PanP) is supported by a high bootstrap value in the NJ and pars methods (80–100%). Using the larger 710 nt consensus fragment, but with only STLV PP1664 as a representative of PTLV-PP, this separation is highly significant (100% of the bootstrap replicates in NJ and pars, P < 0.001 in ML). These analyses favour a fourth type of PTLV, PTLV-PP.

Using almost the entire tax sequence, three subtypes of HTLV-I become apparent (Fig. 4). These correlate with the three major clades identified within the known strains of HTLV-I: a cosmopolitan HTLV-Ia originating from western Africa, a Zairian HTLV-Ib and a Melanesian HTLV-Ic (Koralnik et al., 1994; Vandamme et al., 1994). In previous phylogenetic analyses using the LTR sequence (Vandamme et al., 1994), we argued in favour of an African origin for PTLV-I but could not reject an Asian origin. In our present analysis of the 710 nt tax/rex region (Fig. 4), the Asian strains branched off before the African and cosmopolitan strains with high bootstrap values (100%), suggesting the most ancient lineage of PTLV-I to be in Asia. However, no divergent African STLV-I sequences were available in this region. More data need to be accumulated to solve the origin of PTLV-I.

In all phylogenetic analyses concerning HTLV-II, the two previously reported clades (Hall et al., 1992) clearly emerge: HTLV-IIa and HTLV-IIb. Both subtypes have been identified in the Americas, including indigenous Amerindian tribes (Dube et al., 1993; Ferret et al., 1993; Ishak et al., 1995). Also, in Africa, both HTLV-IIa and HTLV-IIb are present (Gessain et al., 1994, 1995; Igarishi et al., 1993). HTLV-II has also been found in Mongolia (Neel et al., 1994). A single report describes an STLV-II strain, SM-3, found in American spider monkeys (Ateles fusciceps), which has a tax/rex sequence (120 nt available) that is very similar to the corresponding sequence in HTLV-IIa Mo (Chen et al., 1994). However, this sequence has two frame shifts in both Tax and Rex, which would probably render these essential proteins non-functional. Moreover, no additional sequence data are available for this strain. Further evidence is therefore needed to properly assess the presence of STLV in New World monkeys. Since the STLV PP1664 described here is a rather close relative to HTLV-II and has been found in African bonobos, it is more likely that PTLV-II is of African descent.

BLV is the only known non-primate virus in the PTLV/BLV family. PTLV and BLV thus evolved from a common ancestor. Ancient close contacts between humans and cattle were possible both in Africa and in Asia (Loftus et al., 1994). However, since cattle were domesticated only 8–10000 years ago, and the divergence among STLVs is larger than the divergence among HTLVs, it is very unlikely that HTLV has evolved from BLV with a subsequent spread to simians but rather that humans have been infected by simian viruses. Since there is very little sequence similarity between BLV and PTLV, it is very tricky to root the PTLV tree with BLV. Using the only detectable homologous amino acid stretch in Tax, we attempted to align the corresponding nucleotide sequences and to use this alignment in a rooting process. In this short stretch of the tax/rex region, PTLV-I seems to be the most divergent type. It branches off closest to the root, implying an old origin. According to this tax/rex analysis, PTLV-L might have emerged somewhat later. PTLV-II and PTLV-PP have split more recently and could therefore be called the youngest branches. Phylogenetic analyses of other gene regions have identified PTLV-L as the oldest type (Van Brussel et al., 1996). Therefore, it is not clear yet whether PTLV-I or PTLV-L is the most ancient type.

With the new data reported here one could speculate on the following hypothesis for the origin and development of PTLVs. Both Asia and Africa harbour very divergent PTLV-I strains, and PTLV-L has been found in a baboon in East Africa. HTLV-II is endemic in
African and American endogenous populations, but its closest simian relative is the African STLV PP. Therefore the most probable origin of PTLV is in Africa. PTLV-I could have split off very early from the ancestral PTLV and spread simultaneously to Asia and over Africa. PTLV-I was passed between simian species and from simians to humans several times and separately in Asia and in Africa. A PTLV-II-like lineage possibly split from the ancestral PTLV in Africa and diverged into PTLV-PP and PTLV-II. HTLV-II probably emerged in Africa, migrated to Asia and passed the Bering Strait along with its human hosts. In this scenario it is difficult to explain the near absence of HTLV-II in Asian populations. Possibly, the virus gradually disappeared from Asia, leaving only some scattered remnants in indigenous African and Amerindian tribes. The PTLV-II sequences tend to have the most recent evolution, which could explain their low genetic divergence. Similarly, the relatively high genomic divergence within PTLV-I is in agreement with its presumed old origin. It is at present not known whether the PTLV-I or PTLV-PP types will have human representatives. Possible human members of the PTLV-I and PTLV-PP types could have stayed unnoticed, since they would be judged as indeterminate, a test result that is often seen in HTLV screening procedures.

With the discovery of a new PTLV type, PTLV-L, in an African baboon, which is as divergent from PTLV-I and PTLV-II as both are from each other, the African bonobo virus reported here further widens the classification of PTLV. At this time we cannot predict whether the four PTLV types described will remain four separate clusters, or if new intermediate PTLV isolates or additional clusters will be discovered. The genetic diversity of PTLV strains may eventually resemble the HIV/SIV genetic variation, which has at present five major genotypes, with several subtypes sequenced within these genotypes (Louwagie et al., 1993; Myers et al., 1994; Sharp et al., 1994). Only the time scale is different, with centuries for HIV/SIV to develop a high diversity, and tens of thousands of years for PTLV.

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