VIROLOGY 251, 71–84 (1998)
ARTICLE NO. VY989377

Evolutionary Inferences of Novel Simian T Lymphotropic Virus Type 1 from Wild-Caught Chacma (*Papio ursinus*) and Olive Baboons (*Papio anubis*)

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Received May 8, 1998; returned to author for revision June 29, 1998; accepted August 12, 1998

A serological survey of 22 wild-caught South African (Transvaal) chacma baboons (*Papio ursinus*) and eight olive baboons (*Papio anubis*) from Kenya indicates that 13 *P. ursinus* and one *P. anubis* have antibodies reacting with human T cell leukemia/lymphoma virus type 1 (HTLV-1) antigens, whereas three *P. ursinus* had a indeterminate reactivity on Western blot analysis. With six primer sets specific to either HTLV-1–Simian T-cell leukemia virus type 1 (STLV-1) or HTLV-2 and encompassing long terminal repeat (LTR), *gag*, *pol*, *env*, and *tax* sequences, polymerase chain reaction was performed on genomic DNA from peripheral blood mononuclear cells of 18 animals, and the presence of HTLV-1–STLV-1-related viruses was determined in 13 seropositive and three seroindeterminate animals but not in the two HTLV seronegative individuals. Proviral DNA sequences from *env* (522 bp), *pol* (120 bp), and complete (755 bp) or partial (514 bp) LTR were determined for three STLV-1-infected *P. ursinus* and one *P. anubis*. Comparative and phylogenetic analyses revealed that *P. anubis* (Pan-486) sequence clusters with one (Pan-1621) of two previously described *P. anubis* STLV-1. Likewise, *P. ursinus* viruses (Pur-529, Pur-539, and Pur-543) form a distinct group, different from all known HTLV-1 but closely affiliated with two STLV-1 strains from South African vervets (*Cercopithecus aethiops pygerythrus*). This study, reporting the first STLV-1 sequences from wild-caught *P. ursinus* and *P. anubis*, corroborates the hypothesis of cross-species transmissions of STLV-1 in the wild. Further, phylogenetic analyses indicate that the known HTLV-1 strains do not share a common origin with nonhuman primates STLV in South Africa.

INTRODUCTION

The primate T lymphotropic viruses (PTLVs) (Guo *et al.,* 1984; Watanabe *et al.,* 1985, 1986) include human T cell leukemia virus type 1 (HTLV-1) (Poiesz *et al.,* 1980; Yoshida *et al.,* 1982), Simian T cell leukemia virus type 1 (STLV-1) (Miyoshi *et al.,* 1983), HTLV-2 (Kalyanaraman *et al.,* 1982), and the recently discovered primate T cell leukemia virus type L (PTLV-L) (Goubau *et al.,* 1994; Van Brussel *et al.,* 1997), as well as the HTLV-2-related $STLV_{Pan\text{-}p}$ (Digilio *et al.,* 1997; Giri *et al.,* 1994; Liu *et al.,* 1994b; Vandamme *et al.,* 1996) and constitute a group of related exogenous retroviruses. HTLV-1 is the etiological agent of a malignant CD4 lymphoproliferation [adult T cell leukemia (ATL)] (Seiki *et al.,* 1983; Yoshida *et al.,* 1982) and of a chronic progressive neuromyelopathy [tropical spastic paraparesis–HTLV-1-associated myelopathy (TSP/HAM)] (Gessain *et al.,* 1985), whereas STLV-1 can cause an ATL-like pathology in infected monkeys. Several seroepidemiological studies of captive monkeys indicate that many species of Old World monkeys, such as macaque, chimpanzee, gorilla, grivet, cercopithecus, and baboon, are STLV-1 carriers (Fultz 1994; Ibrahim *et al.,* 1995; Ishikawa *et al.,* 1987). In contrast, little is known about viral prevalence in wild-caught Simian species (Durand *et al.,* 1995; Fultz 1994; Voevodin *et al.,* 1996a, 1997a).

The molecular epidemiology of HTLV-1 proviruses indicates that the few nucleotide substitutions observed among strains are specific to the geographic origin of the patient but are unrelated to viral pathology (Daenke *et al.,* 1990; Gessain *et al.,* 1992; Komurian-Pradel *et al.,* 1991; 1992; Mahieux *et al.,* 1995, 1997a). A consensus of phylogenetic analyses of sequence and/or restriction fragment length polymorphism data from *pol* and *env* genes as well as from the long terminal repeat (LTR) of >250 different HTLV-1 strains has established four major geographic subtypes: Cosmopolitan, HTLV-1 subtype A (Gessain *et al.,* 1992; Miura *et al.,* 1994); Central African, HTLV-1 subtype B (Fukasawa *et al.,* 1987; Gessain *et al.,* 1992; Liu *et al.,* 1994a; Paine *et al.,* 1991; Ratner *et al.,* 1985); Melanesian, HTLV-1 subtype C (Gessain *et al.,* 1991, 1993; Saksena *et al.,* 1992; Song *et al.,* 1994; Yanagihara, 1994); and the recently discovered subtype D (Chen *et al.,* 1995; Mahieux *et al.,* 1997a; Moudjeka *et al.,* 1997; Moynet *et al.,* 1995). The origin of geographic subtypes appears to be linked with episodes of interspecies transmission between STLV-1-infected monkeys and humans, followed by variable periods of evolution in the human

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0042-6822/98 $25.00

host (Franchini *et al.,* 1995; Giri *et al.,* 1997; Ibrahim *et al.,* 1995; Koralnik *et al.,* 1994; Liu *et al.,* 1996; Mahieux *et al.,* 1997a; Saksena *et al.,* 1994; Song *et al.,* 1994; Vandamme et al., 1994; Watanabe *et al.,* 1985, 1986). However, clear evidence of interspecies transmission consists only of a described affiliation between HTLV-1 subtype B from Central African inhabitants and STLV-1 from chimpanzees (Koralnik *et al.,* 1994; Mahieux *et al.,* 1997a; Voevodin *et al.,* 1997b). Recently, sequence analyses suggest that molecular subgroups, defined by several specific mutations, exist within the four main genotypes. However, these subgroups are not always recapitulated by phylogenetic analyses (Mahieux *et al.,* 1997a).

Although serological data have been available for years (Becker *et al.,* 1985; Bhigjee *et al.,* 1993), data on the molecular epidemiology of HTLV-1 in South Africa are relatively recent. HTLV-1 strains from this country are seemingly associated with the Cosmopolitan A subtype (Engelbrecht *et al.,* 1996; Mahieux *et al.,* 1997a; Yamashita *et al.,* 1995b). Although still a matter of debate (Yamashita *et al.,* 1995b), HTLV-1 presumably entered this area during the historic Bantu expansion of the early and late Iron Age (1500–2000 years ago) (Cavalli-Sforza *et al.,* 1994). In contrast, analysis of STLV-1 sequences obtained from two infected South African vervet monkeys (*Cercopithecus aethiops pygerythrus*) suggests these simian viruses are related to the Central African STLV-1 group (Engelbrecht *et al.,* 1996).

Among species of STLV-1-infected primates, the situation of baboons (genus *Papio*) is unique and complex (Voevodin *et al.,* 1997a). Analysis of viral sequences indicates *P. hamadryas* are carriers in the wild of at least two types of PTLV: an HTLV-1-B-related virus (Liu *et al.,* 1997; Voevodin *et al.,* 1996a) and the divergent PTLV-L (Goubau *et al.,* 1994). Other baboon species, such as *P. cynocephalus* (Guo *et al.,* 1984; Voevodin *et al.,* 1997a), *P. papio*, and *P. anubis* (Koralnik *et al.,* 1994; Vincent *et al.,* 1996), harbor STLV-1 as well. Evidence of possible cross-species transmission occurring either by chance or after blood product inoculation in primatology centers (Voevodin *et al.,* 1996b) is indicated because viral strains from *P. cynomolgus* and *P. hamadryas* STLV-1 are identical to true macaque (*Macaca mulatta*) STLV-1. In some cases, it is virtually impossible to distinguish *P. anubis* viral sequences from that of *P. hamadryas* or *P. cynocephalus* STLV-1 (Koralnik *et al.,* 1994). Initially interpreted as evidence of STLV-1 interspecies transmission in the wild, these episodes more likely occurred during captivity (Mone *et al.,* 1992). Therefore the natural incidence of interspecies transmission of STLV-1 between baboons and other primates has yet to be confirmed. Consequently, the goals of the present study are (1) to isolate and characterize previously undescribed STLV-1 obtained from natural populations of wild-caught individuals of *P. ursinus* dwelling in South Africa; (2) to characterize genetically STLV-1 sequences from a wild-caught *P. anubis,* a species for which only partial viral sequences are available from two captive animals; and (3) to provide evidence of interspecies transmissions between human and nonhuman primates from the same geographic area.

RESULTS

Identification of HTLV-1/STLV-1 infection in the two different baboon subspecies by detection of specific HTLV-1–STLV-1 antibodies

The sera of 30 wild-caught animals were tested by immunofluorescence assay (IFA) for the presence of STLV-1 antibodies (Table 1). Sixteen of 22 (73%) *P. ursinus* and one of eight (12.5%) *P. anubis* exhibited HTLV-1–STLV-1 antibodies reacting with HTLV-1 antigens produced by MT-2 cells as well as HTLV-2 antigens produced by C19 cells. However, in all cases, the antibody titer was higher on MT-2 cells (median 1:320, range 1:40 to 1:2560) than on C19 (median 1:40, range 1:20 to 1:320). Such data indicated that these animals were infected with an HTLV-1–STLV-1-related virus rather than with an HTLV-2-related virus. Thirteen *P. ursinus* and one *P. anubis* further exhibited strong and complete positive HTLV-1–STLV-1 Western blot seroreactivities, whereas three *P. ursinus* sera were found as seroindeterminate (Table 1). In the latter, one case exhibited all reactivities except the MTA-1 band; the second showed all the bands except p24 and MTA-1; and the third exhibited only GD21, p19, and MTA-1 (Fig. 1). The remaining 14 sera were all negative in Western blot assays.

Confirmation of HTLV-1–STLV-1 infection by PCR

For polymerase chain reaction (PCR) analysis, DNA from peripheral blood mononuclear cells of 16 *P. ursinus* and two *P. anubis* representing 13 seropositive animals, three seroindeterminate and two HTLV seronegative ones were assayed (Table 2). In all seropositive animals and the three seroindeterminate animals, the *env* and *tax* PCR detected HTLV-1–STLV-1-related DNA sequences, whereas the HTLV-2 primer sets and probes were negative. The HTLV-1–STLV-1 *gag* and *pol* PCRs were slightly less sensitive and did not amplify viral DNA in one animal (Table 2). No HTLV-1–STLV-1 DNA sequences were detected in either of the two seronegative animals or in the negative controls.

DNA sequence analyses of the complete LTR region and of partial *env* and *pol* genes from *P. ursinus* and *P. anubis* STLV-1

Three genomic fragments from the proviral DNA of the STLV-1 present in three *P. ursinus* and one *P. anubis*

TABLE 1

Species, Sex, Age, Origin, and HTLV Serological Status of the 30 Baboons Tested

Baboon no.	Species	Sex	Age	Origin	IFA titer MT2	IFA titer C19	WB
526	PUR	M	>15	South Africa	1/160	1/40	+
528	PUR	M	>15	South Africa	1/320	1/40	+
529	PUR	M	>15	South Africa	1/160	1/40	+
530	PUR	M	>15	South Africa	1/640	1/80	ind[a]
531	PUR	M	>15	South Africa	1/80	1/40	+
532	PUR	M	>15	South Africa	1/640	1/40	+
533	PUR	M	>15	South Africa	1/80	1/40	+
535	PUR	M	>15	South Africa	1/320	1/80	ind[b]
536	PUR	M	>15	South Africa	1/640	1/40	+
537	PUR	M	>15	South Africa	1/320	1/80	+
539	PUR	M	>15	South Africa	1/80	1/20	+
541	PUR	M	>15	South Africa	1/40	1/20	+
543	PUR	M	>15	South Africa	1/2560	1/320	+
545	PUR	M	>15	South Africa	1/20	1/20	ind[c]
546	PUR	M	>15	South Africa	1/320	1/80	+
C9A	PUR	M	>15	South Africa	1/640	1/160	+
486	PAN	M	>15	Kenya	1/60	1/40	+
527	PUR	M	>15	South Africa	0	0	−
534	PUR	M	>15	South Africa	0	0	−
538	PUR	M	>15	South Africa	0	0	−
540	PUR	M	>15	South Africa	0	0	−
542	PUR	M	>15	South Africa	0	0	−
544	PUR	M	>15	South Africa	0	0	−
180	PAN	M	>15	Kenya	0	0	−
438	PAN	M	>15	Kenya	0	0	−
470	PAN	M	>15	Kenya	0	0	−
474	PAN	M	>15	Kenya	0	0	−
482	PAN	M	>15	Kenya	0	0	−
483	PAN	M	>15	Kenya	0	0	−
484	PAN	M	>15	Kenya	0	0	−

[a] Lack of p24 and of MTA-1 reactivities.
[b] Lack of MTA-1 reactivity.
[c] Presence of only GD-21, MTA-1, and p19 bands.

were studied: the complete LTR (755 bp) in three cases or a fragment of 514 bp in one case, a 522-bp fragment of the *env* gene, and a 120-bp fragment of the *pol* gene.

Sequence analysis of a fragment of gp21 *env*

A comparison of the aligned 522-bp fragments indicated no deletions or insertions as compared to the ATK reference sequence. Genetic comparison of three *P. ursinus* and the *P. anubis env* sequences using a fragment of 387 bp for all published STLV-1 *env* sequences from baboons (Table 3) suggested that Pur-529, Pur-539, and Pur-543 were closely related to each other (99.2–99.5% similarity) but distinct from Pan-486 (95–95.9% of similarity). Moreover, STLV-1 from *P. ursinus* do not share significant sequences similarities with any other previously reported baboon STLV-1 viruses isolated either from *P. hamadryas, P. cynocephalus*, or *P anubis.* On the contrary, the Pan-486 sequence was more similar to one of two previously known *P. anubis,* sharing a similarity of 98.9% with Pan-1621 (Vincent *et al.,* 1996) and 96.4% with Pan-1713 (Koralnik *et al.,* 1994).

Sequence analysis of a fragment of *pol*

Despite the fact that this small fragment is not sufficient to separate clearly HTLV-1 and STLV-1 clades within a molecular/geographic subtype, we performed sequence analyses of this short fragment because this is the only one available for some monkeys from West and Central Africa. No differences were assayed among the three new *P. ursinus* viruses and those from the three HTLV seroindeterminate animals (Pur-530, Pur-535, and Pur-435) (data not shown). Comparison with *P. anubis* yielded a similarity of 96.7% and values of 94.2–97.5% for other *Papio* STLV-1 and 91.2–97.5% for other Old World species. On the contrary, the Pan-486 sequence was very closely related (99.2%) to Pcy-2304 and Pha-152, two STLV-1 isolates from a *P. cynocephalus* and a *P. hamadryas,* respectively, kept at the baboon colony of Southwest

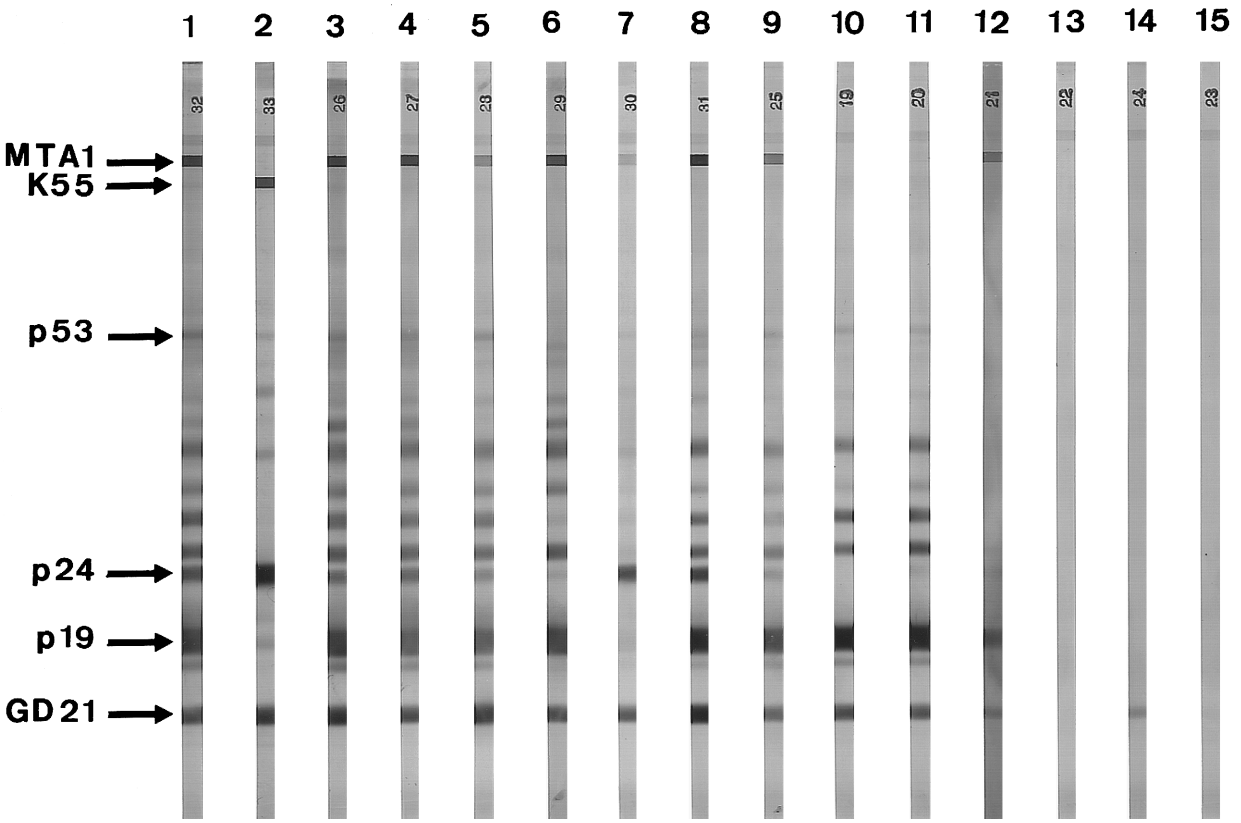

FIG. 1. Western blot analysis. The Western blot was from Diagnostic Biotechnology (HTLV-blot 2–4). Lane 1 indicates HTLV-1-positive control; lane 2, HTLV-2-positive control; lanes 3–8, six sera samples from STLV-1-infected *P. ursinus* with complete HTLV-1 seroreactivity, lane 9, sera from a *P. anubis* with a complete HTLV-1 seroreactivity; lane 10, sera from a *P. ursinus* with a complete pattern except a reactivity against the p24 and the MTA-1 peptide; lane 11, sera from a *P. ursinus* with a complete pattern except a reactivity against the MTA-1 peptide; lane 12, sera from a *P. ursinus* with reactivities against only GD21, p19, and MTA-1; lanes 13 and 15, sera from three HTLV-1–STLV-1-seronegative *P. ursinus*; and lane 14, sera from a *P. ursinus* with a faint reactivity only against GD21; such sera are usually considered as negative.

Foundation for Biomedical Research (San Antonio, TX) (Voevodin *et al.,* 1997a) (Table 4).

Sequence analysis of the LTR

The LTR, especially in the U3 and U5 portions, is a highly variable fragment that is phylogenetically informative among HTLV–STLV strains. Despite the fact that only 44 complete or ~90 partial (large fragment of >600 bp) LTR sequences are available, compared with >150 for the gp21 *env* region, some HTLV-1–STLV-1 strains have been sequenced only in this region. Thus a more complete phylogenetic analysis of our new baboon STLV-1 encompassed additional strains identified by LTR variation.

In the complete LTR analysis, Pur-529 and Pur-543 were more closely related (98.7% similarity) than with Pan-486 (95.3% and 95.7%, respectively) (Table 5). Shorter fragments of the LTR, obtained for Pur-530 and Pur-535, which exhibited an HTLV indeterminate seroreactivity by WB, were nearly identical to the equivalent region of Pur-529 and Pur-543. Furthermore, no other previously published LTR STLV-1 sequences exhibited a high degree of similarity with the three new baboon sequences.

In the U3 region, three *tax*-responsive elements (TREs) with a conserved central core and the *c-ETS*-responsive elements were conserved. In the R region, containing the *rex*-responsive element, a high degree of similarity was observed in the stem sequence (suggesting that its functionality is maintained), as well as in the *rex* binding region between STLV-1 from Pur-529, 539, 543, Pan-486, ATK, and other available STLV-1 LTR sequences.

Phylogenetic analyses

The evolutionary relationships of the new baboon viral sequences relative to previously described genetic variants were examined with three different sequence segments: the complete LTR, a partial fragment of the LTR, and the *env* gp21 gene. The resultant alignments of 44 complete LTR (755 bp) sequences, of 65 partial LTR sequences (514 bp) (Fig. 2), and 93 sequences of *env* gp21 (522 bp) (Fig. 3) were analyzed by both maximum parsimony (MP) and neighbor-joining (NJ) phylogenetic algorithms.

TABLE 2

Detection of STLV-1 *gag*, *pol*, *env*, *tax*, or LTR Sequences by PCR in the PBMCs of 18 Different Baboons after Hybridization with γ ^{32}P-Labeled Internal Specific Probes

Baboon no.	Gag[a]	Pol[a]	Pol[b]	Env[a,d]	Tax[a]	LTR[a,d]
526 PUR	−	+	−	+	+	ND
528 PUR	+	+	−	+	+	ND
529 PUR	+	+[c]	−	+[c]	+	+[c]
530 PUR	+	+[c]	−	+	+	+[c]
531 PUR	+	−	−	+	+	ND
532 PUR	+	+	−	+	+	ND
533 PUR	+	+	−	+	+	ND
535 PUR	+	+[c]	−	+	+	+[c]
537 PUR	+	+	−	+	+	ND
539 PUR	+	+[c]	−	+[c]	+	+[c]
540 PUR	−	−	−	−	−	ND
541 PUR	+	+	−	+	+	ND
543 PUR	+	+[c]	−	+[c]	+	+[c]
545 PUR	+	+[c]	−	+	+	ND
546 PUR	+	+	−	+	+	ND
C9A PUR	+	+	−	+	+	ND
482 PAN	−	−	−	−	−	ND
486 PAN	+	+[c]	−	+[c]	+	+[c]

Note. ND, not done.
[a] Primers specific for HTLV-1-STLV-1.
[b] Primers specific for HTLV-2.
[c] Sequence obtained.
[d] Nested PCR.

Phylogenetic reconstruction with LTR sequences supported several well-defined evolutionary clusters (Fig. 2). Rooted by divergent strains of HTLV-1 from Melanesia, STLV-1 from Asian macaques [Mto-TE4 (*M. tonkeana*) and Mne-PTM-3 (*M. nemestrina*)], the remaining viral strains form a distinct bifurcation into two major groups (or clades). One group consisted of a global assemblage of human viruses corresponding to subtype A with bootstrap support of 79% (NJ) and 57% (MP). However, the relative internal branching order uniting these strains remained ambiguous. The other group was composed of human (HTLV-1 subtypes B and D) strains interleaved with simian viruses from Africa. Within each of the two major groups, distinct monophyletic subclades, of human or simian (or both) species viruses, were identified that shared a common geographic locale.

The three newly described *P. ursinus* viruses (Pur-539, Pur-529, Pur-543) from South Africa form a cluster with high bootstrap support (86% NJ, 80% MP) that is affiliated with the monophyletic lineage composed of two South African vervets (*Cercopithecus aethiops pygerythrus*) (Cae-tbhv191, Cae-tbhv197) (Fig. 2). Basal to this South African Simian lineage are two viruses: one from a baboon, *P. cynocephalus* from Tanzania (Pcy-1011), and the other from a *C. aethiops* from South Africa (Cae-AG). Although maximum parsimony and neighbor-joining methods unite these latter two species with the South African cluster, only maximum parsimony yielded strong bootstrap support (82%). Neighbor-joining differs by placing the Pcy-1011 sequence within this group (76%) but does not support the placement of Cae-AG in this cluster.

The newly described baboon sequence from Kenya (Pan-486) was not clearly affiliated with any group. In the maximal parsimony analysis, this strain was loosely associated with the African STLV clade. In contrast, the NJ tree placed Pan-486 in association with the Central African type B HTLV strains (Fig. 2).

Other well-supported subgroups within HTLV-1 Cosmopolitan subtype A demonstrates the geographic basis of viral evolution. Subgroups from Japan, North Africa, Ivory Coast, South America, and South Africa were clearly distinguished by identical topology and favorable bootstrap proportions between MP and NJ analyses. Clades within the type B (Central African) HTLV-1 was less distinct with MP analysis. However, NJ analysis separated the HTLV B and HTLV D subgroups. Analysis of the complete LTR of a subset of 43 sequences recapitulated the known subtypes A, B, C, and D with high bootstrap values for each (data not shown). Further, the complete LTR analyses confirmed both the association of *P. ursinus* viruses with Cae-AG (Kenya) and Cae-tbhv (South Africa) and the lack of strong affiliation with Pan-486.

With the 93 *env* sequences, phylogenetic analysis of the baboon sequences included a representative of previously described HTLV-1–STLV-1. Both MP and NJ trees exhibit similar topologies with monophyletic subgroups composed of Simian viruses collected from the same geographic regions and HTLV-1, forming broader geo-

TABLE 3

Genetic Divergence Among All Pairs of Viral Sequences in the 387 bp *env* Region of All Known Baboon STLV-I Sequences

	Pha 152[a]	Pha PH6356[b]	Pha Su-F1[c]	Pan 5 × 28[a]	Pur 529[d]	Pur 539[d]	Pur 543[d]	Pan 486[d]	Pan 1621[e]	Pcy 2029[f]	Pcy 3003[f]	Pcy kz05[f]	Pcy 5101[f]	Pcy 1011[f]	Pcy 2304[a]	Pcy 991[ai]	Pha Su-L2[gi]	Pha Su-L1[gi]	Pha PHSu1[hi]	Pha su5945[gi]
Pan 1713	99.5	95.6	95.1	94.8	96.4	97.2	96.7	96.4	95.9	96.4	96.1	96.7	96.4	96.4	99.5	86.3	86.8	85.8	86.8	86.8
Pha 152		95.6	95.1	94.8	96.7	97.4	96.9	96.4	95.9	96.9	96.6	97.2	96.9	96.9	100	86.3	86.9	85.8	86.8	86.8
Pha PH6356			97.4	97.1	94.8	95.6	95.6	98.2	98.7	94	93.8	94.3	94	94	95.6	87.8	89.6	87.6	88.4	88.4
Pha Su-F1				97.1	94.8	95.6	95.6	97.7	97.7	93.8	93.5	94	93.8	93.8	95.1	87.1	89	86.8	87.6	87.6
Pan 5 × 28					93.5	94.3	94.3	97.9	97.9	93.5	93.3	93.3	93	93	94.8	87.6	88.1	87	88.1	88.1
Pur 529						99.2	99.2	95	94.6	95.1	94.8	95.4	95	95	96.7	85.5	85.8	84.5	85.8	85.8
Pur 539							99.5	95.9	95.4	95.3	95	96.1	95.9	95.9	97.5	86.6	86.9	85.5	86.9	86.9
Pur 543								95.4	95.4	95.9	95.6	95.6	95.4	95.4	96.9	86	86.3	85	86.3	86.3
Pan 486									98.9	95	94.8	94.9	94.6	94.6	96.4	88.1	88.7	87.6	88.6	87
Pan 1621										94.6	94.3	94.3	94	94	95.9	88.1	88.4	87.4	88.4	88.4
Pcy 2029											99.7	99.7	100	100	96.9	86	85.3	84.3	85.3	86.3
Pcy 3003												99.5	99.7	99.7	96.6	85.8	88.4	85.3	86	86
Pcy kz05													99.8	99.8	97.2	85.3	85.5	84.5	85.5	85.5
Pcy 5101														100	96.9	85	85.3	84.3	85.3	85.3
Pcy 1011															96.9	85	85.3	84.3	85.3	85.3
Pcy 2304																86.3	86.9	85.8	86.8	86.9
Pcy 991																	99.2	98.2	99.2	99.2
Pha SuL2																		99.2	100	100
Pha SuL1																			99.2	99.2
Pha PHSu1																				100

Note. Pha, *P. hamadryas;* Pan, *P. anubis;* Pur, *P. ursinus;* Pcy, *P. cynocephalus.*

[a] Koralnik *et al.* (1994).

[b] Liu *et al.* (1997).

[c] Voevodin *et al.* (1996a).

[d] This study.

[e] Vincent *et al.* (1996).

[f] Voevodin *et al.* (1997a).

[g] Voevodin *et al.* (1996b).

[h] Liu *et al.* (1996).

[i] Baboons (*P. hamadryas*) carriers of macaques (*M. mulatta*) STLV-1 viruses.

graphic subtypes (Fig. 3). Bootstrap support for these groups varies, yet these are consistencies between the two methods. Major evolutionary lineages recapitulated included four recognized subtypes of HTLV-1. Interspersed with these evolutionary groups were STLV-1 sequences from local regions throughout Africa. The three new STLV-1 sequences from South African baboons (Pur-529, Pur-543, Pur-539) formed a distinct monophyletic group (bootstrap proportions of 100% NJ, MP) apart from any other viral strain. Similarly, the new baboon virus, Pan-486 from Kenya, formed a distinct group with a strain previously identified from the same species of baboon (Pan-1621) from Kenya along with the STLV from another baboon species, *P. papio* from West Africa (Ppa-5X28),

DISCUSSION

Previous studies describing a close evolutionary relationship uniting some African STLV-1 isolates from different species with some HTLV-1 strains (Koralnik *et al.,* 1994; Liu *et al.,* 1996, 1997; Mahieux *et al.,* 1997a; Song *et al.,* 1994; Voevodin *et al.,* 1997a, 1997b) formed the basis to the concept of interspecies viral transmission among primates, including humans. However, for some simian species, simian species, recent data suggest the possibility that these transmission episodes occurred after capture (Voevodin *et al.,* 1996b). Consequently, it remained unclear whether natural STLV-1 isolates existed in the wild, especially with the genus *Papio* (baboons). Therefore this study aimed to genetically characterize the first STLV-1 sequences from wild-caught *P. ursinus* (from South Africa) and *P. anubis* [living in East Africa (Kenya)] and to compare them with HTLV-1 and STLV-1 from these regions. The possibility of episodes of iatrogenic, nosocomial, or experimental cross-species transmission was excluded by using samples directly from wild-caught animals. Thus these isolates may offer the best representation of naturally occurring STLV-1 in *P. ursinus* and *P. anubis* troops.

The importance of using different regions of the viral genome in phylogenetic analyses was evident from the interferences derived from the LTR versus the *env* gene. Nearly all STLV-1 relationships described previously (Koralnik *et al.,* 1994; Liu *et al.,* 1996, 1997; Mahieux *et al.,*

TABLE 4

Genetic Divergence among All Pairs of Viral Sequences in the 120-bp *pol* Region of All Known Baboon STLV-1 Sequences

	Pan 486[a]	Pur 529[a]	Pur 539[a]	Pur 543[a]	Pcy KIA[b]	Pcy 1011[c]	Pcy 2304[c]	Pcy 3003[c]	Pcy 5101[c]	Pcy KZ05[c]	Pha Su-F1[d]	Pha 152[c]	Pdo BAB34[e]
Pan 486		96.7	96.7	96.7	98.3	97.5	99.2	97.5	97.5	97.5	95.8	99.2	95
Pur 529			100	100	96.7	96.7	97.5	96.7	96.7	96.7	94.2	97.5	93.3
Pur 539				100	96.7	96.7	97.5	96.7	96.7	96.7	94.2	97.5	93.3
Pur 543					96.7	96.7	97.5	96.7	96.7	96.7	94.2	97.5	93.3
Pcy KIA						97.5	99.2	97.5	97.5	97.5	95.8	99.2	93.3
Pcy 1011							98.3	100	100	100	95	98.3	95
Pcy 2304								98.3	98.3	98.3	96.6	100	94.2
Pcy 3003									98.3	100	95	98.3	95.8
Pcy 5101										100	95	98.3	94.2
Pcy KZ05											98.3	98.3	94.2
Pha Su-F1												96.7	94.2
Pha 152													92.5
Pdo BAB34													95.8

Note. Pha, *P. hamadryas;* Pan, *P. anubis;* Pur, *P. ursinus;* Pcy, *P. cynocephalus;* Pdo, *P. doguera.*
[a] This study.
[b] Song *et al.* (1994).
[c] Voevodin *et al.* (1997a).
[d] Voevodin *et al.* (1996a).
[e] Saksena *et al.* (1994).

1997a; Voevodin *et al.,* 1996a, 1997a, 1997b) were recovered in the present phylogenetic analyses, and placement of the new baboon sequences appeared to be correlated with the geographic origin of the strains. LTR sequences were more informative, having other South African STLV-1 sequences available for comparison (Engelbrecht *et al.,* 1996) and because no corresponding *env* samples were available for this geographic region.

The STLV-1 present in the infected *P. anubis* appears to be allied to a previously described isolate from a captive animal of the same species (Pan-1621) (Vincent *et al.,* 1996). Although this result suggests Pan-486 is the natural strain for *P. anubis,* the association remains speculative. No bootstrap support is detected for the Pan-1621 and Pan-486 association. Moreover, no corroboration was possible from the LTR analysis because Pan-1621 was not sequenced in this genomic region. The absence of close similarity of Pan-486 with the only other

TABLE 5

Genetic Divergence among All Pairs of Viral Sequences in the 705-bp LTR Region of All Known Baboon STLV-1 Sequences and Related Vervet Sequences

	Pan 486[a]	Pur 529[a]	Pur 543[a]	Pcy 1011[b]	Pcy 5101[b]	Pcy 2304[b]	Pha SU-F1[c]	Pha PH6356[d]	Tbh V-191[e]	Tbh V-197[e]	Pha PHSU1[fg]
Pan 486		95.3	95.7	95.45	96	95.7	95.9	95.2	95	93	87.4
Pur 529			98.7	95.4	95.9	96.1	95.4	95	96.9	95.3	87
Pur 543				95.3	95.9	96	95.6	95	96.7	95.3	86.3
Pcy 1011					99.4	95.9	94.7	93.6	94.4	93.2	86.6
Pcy 5101						96.5	95.3	94.2	95	93.75	87.2
Pcy 2304							95.3	94.3	96.1	94.4	86.6
Pha SUF-1								97.3	94.7	93.5	86.5
Pha PH6356									93.4	92.2	85.8
Tbh V-191										95.7	85.5
Tbh V-197											94.7

Note. Pha, *P. hamadryas;* Pan, *P. anubis;* Pur, *P. ursinus;* Pcy, *P. cynocephalus;* Tbh, *C. aethiops pygerythrus.*[a] This study.
[b] Voevodin *et al.* (1997a).
[c] Voevodin *et al.* (1996a).
[d] Liu *et al.* (1997).
[e] Engelbrecht *et al.* (1996).
[f] Liu *et al.* (1996).
[g] Baboon (*P. hamadryas*) carrier of macaque (*M. mulatta*) STLV-1 virus.

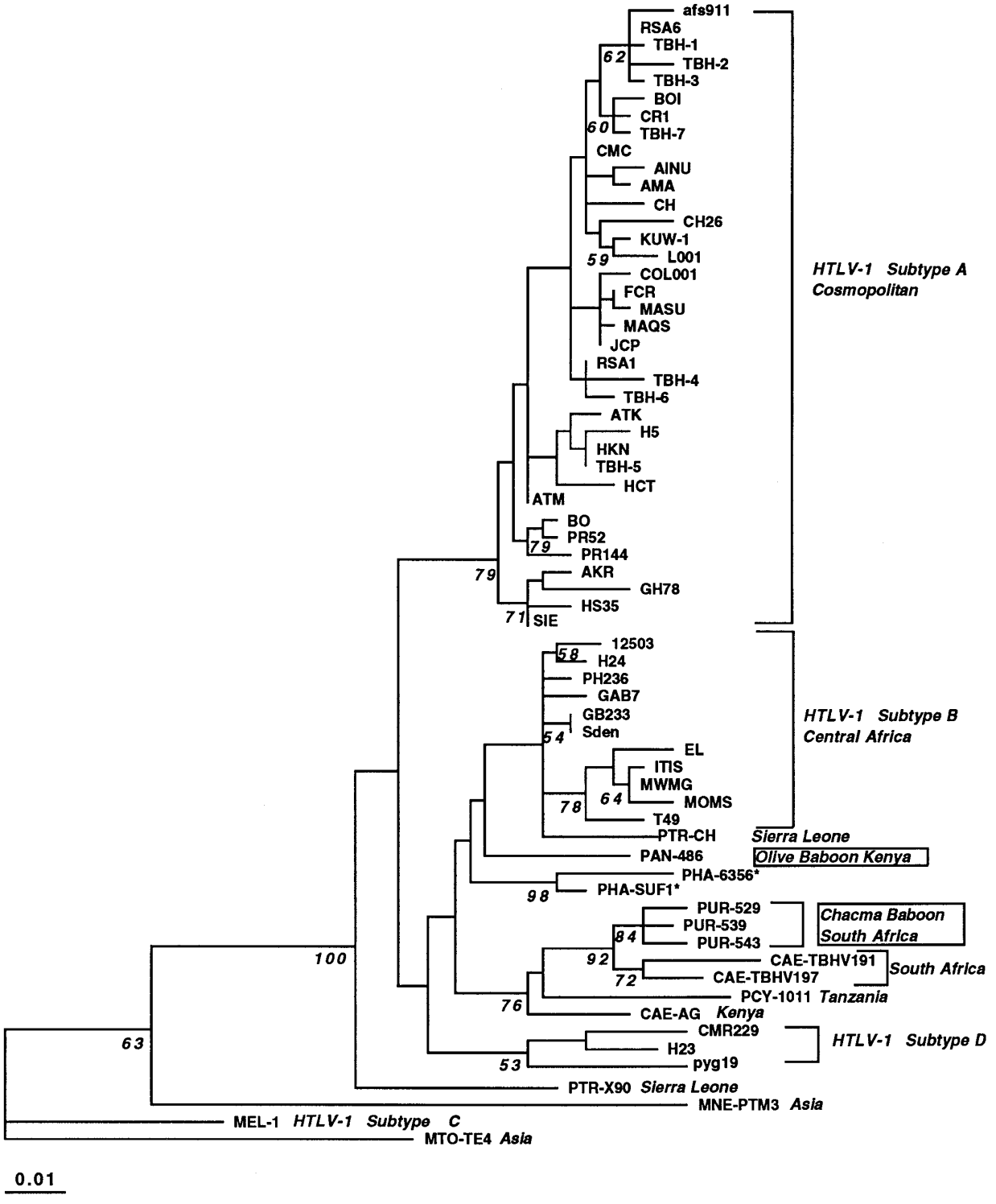

FIG. 2. Phylogenetic tree derived by minimum evolution estimated by neighbor-joining (NJ) using the 65 partial HTLV–STLV LTR sequences (514 bp), including the three new *P. ursinus* and the new *P. anubis* strains. ∗, Sequences of unknown geographic origin. Scale represents percent sequence divergence. Tree was constructed using the Tajima–Nei model of substitution. Numbers in italics represent bootstrap proportions in support of adjacent node. Only bootstrap values of >50% were used in this program. Trees rooted using divergent strain MTO-TE4 from Asia. Nearly identical topology, evolutionary groups, and bootstrap values were obtained by the maximum parsimony (MP) analyses (data not shown). In the MP analysis, a 50% majority rule consensus of 4100 equivalent trees consisted of 910 steps and a consistency index of 0.68. The LTR sequences were obtained from Chen *et al.,* 1995; Engelbrecht *et al.,* 1996; Gasmi *et al.,* 1994; Ibrahim *et al.,* 1995; Josephs *et al.,* 1984; Komurian *et al.,* 1991, 1992; Liu *et al.,* 1994a, 1996, 1997; Mahieux *et al.,* 1997a; Malik *et al.,* 1988; Miura *et al.,* 1994, 1997; Ratner *et al.,* 1991; Saksena *et al.,* 1992, 1993; Seiki *et al.,* 1983; Shimothono *et al.,* 1985; Shirabe *et al.,* 1990; Tsujimoto *et al.,* 1988; Vandamme *et al.,* 1994, 1996; Voevodin *et al.,* 1995, 1996, 1997a; Watanabe *et al.,* 1986; and Yamashita *et al.,* 1995a, 1995b. The geographical origins of the new isolates are boxed.

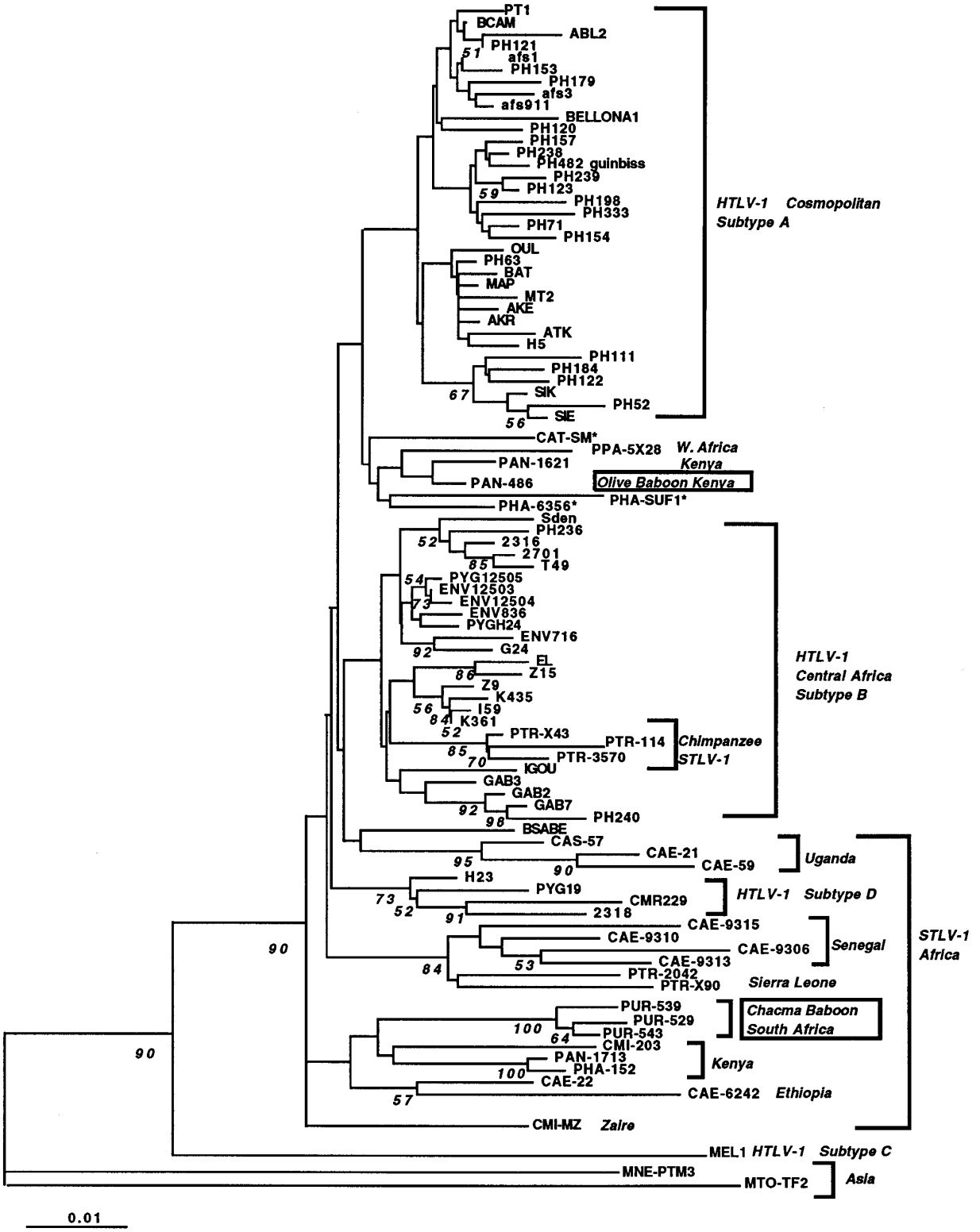

FIG. 3. Phylogenetic tree derived by minimal evolution estimated by neighbor-joining (NJ) using 93 partial HTLV/STLV *env* sequences. This fragment of 522 bp encompasses most of the gp21 and the carboxyl terminus of the gp46. Shown is one of two equivalent trees derived by an heuristic search using the Kimura two-parameter model of substitution. Numbers in italics are bootstrap proportions in support of adjacent node. Only bootstrap values of >50% were used in this program. Trees is rooted by divergent MTO-TF2 from Asia. Asterisk denotes unknown geographic origin of viral strain. Nearly identical topology, evolutionary groups, and bootstrap values were obtained by the maximum parsimony (MP) analyses (data not shown). In the MP analysis, a 50% majority rule consensus of 4100 equivalent trees consisted of 568 steps and consistency index of 0.50. The *env* sequences were obtained from Bazarbachi *et al.,* 1995; Gessain *et al.,* 1991, 1992, 1993; Gray *et al.,* 1990; Koralnik *et al.,* 1994; Liu *et al.,* 1997; Mahieux *et al.,* 1994, 1997a; Mboudjeka *et al.,* 1997; Vincent *et al.,* 1996; Paine *et al.,* 1991; Ratner *et al.,* 1991; Schulz *et al.,* 1991; Seiki *et al.,* 1983; Voevodin *et al.,* 1996, 1997a.

available sequence of STLV-1 from a *P. anubis* (Pan-1713; Koralnik *et al.,* 1994) reinforces the view of the possible acquisition of the latter virus during shared captivity with other *Papio* species (Pcy-2304, Pha-152, Pan-1713) in the SFBR colony (Möne *et al.,* 1192; Voevodin *et al.,* 1997a)

In the case of *P. ursinus,* the three new STLV-1 strains are not closely related to any previously reported baboon strains. Analyses of the LTR indicates a close association, with strong bootstrap support (92% NJ and 97% MP) with two South African monkeys STLV-1 strains obtain from wild-caught vervets (*C. aethiops pygerythrus*) (CAE-tbhv191, CAE-tbhv197) (Engelbrecht *et al.,* 1996). These results are consistent with proposed interspecies transmission between species of primates in the wild. It is worth noting that the STLV-1 baboon strains from South Africa do not cluster with known viral strains present in humans from the same region. These human strains clearly belong to the HTLV-1 Cosmopolitan group (Engelbrecht *et al.,* 1996; Mahieux *et al.,* 1997a; Yamashita *et al.,* 1995b). Thus HTLV-1-infected populations residing in South Africa may have acquired the virus from a different simian reservoir: either a species living currently in the southern part of the African continent (Botha *et al.,* 1985) or earlier, from a species encountered during the Bantous migration into South Africa ~1500–2000 years ago (Cavalli Sforza *et al.,* 1994).

Three *P. ursinus* exhibit an indeterminate HTLV Western blot (WB) (Fig. 1) seroreactivity, but analyses of short regions of the *pol* and LTR reveal all three are identical to those viruses isolated from *P. ursinus* with complete WB patterns. In two cases, the indeterminate pattern of these animals differed by missing either reactivity against the MTA-1 peptide band (Pur-535) or reactivity against MTA-1 and p24 antigen (Pur-530). In the third case (Pur-545), the sera present reactivities only to p19, p24, and MTA-1. Thus detection of STLV-1 sequences in monkeys with incomplete reactivity is consistent with other evidence (Ibrahim *et al.,* 1995).

Ongoing studies aiming to characterize new strains of the HTLV–STLV family, especially from wild-caught African simian species, are crucial in generating new insights into the origin, evolution, and modes of dissemination of these retroviruses and thus open new avenues of research on the coancestry of primate retroviruses and their human hosts.

MATERIALS AND METHODS

Subjects and specimens

Twenty-two adult male chacma (*P. ursinus*) and eight adult male olive wild baboons (*P. anubis*) were captured in 1995–1996 in South Africa (Transvaal area) and Kenya, respectively. All were kept separately and transported to the Service de Biologie Appliquée (Dr. Diane Agay and Dr. Antonia Van Uye, Centre de Recherche, Service de Santé des Armées, Grenoble, France). On arrival, the animals were housed separately to prevent any viral contamination and were never inoculated with any human or simian biological material. Whole blood was obtained by venipuncture from the internal saphenous vein with blood put into Vacutainer tubes with EDTA and then sent to our laboratory for separation into plasma and peripheral blood mononuclear cells (PBMCs).

Serological test

An indirect IFA using MT2 HTLV-1-producing cells and C19 HTLV-2-producing cells was used for screening and for antibody titration as previously described (Mahieux *et al.,* 1997a). For confirmation, a Western blot (immunoblot) assay (HTLV-2–4; Diagnostic Biotechnology) containing disrupted HTLV-1 virions, a recombinant gp21 (GD21) protein, and MTA-1 (an HTLV-1-specific peptide corresponding to residues 169–209 of the gp46 protein) was used (Buckner *et al.,* 1992; Hadlock *et al.,* 1992). Stringent Western blot criteria were defined, and a sample was considered as HTLV-1–STLV-1 positive only by exhibiting antibodies against both p19 and p24 antigens as well as against both GD21 and MTA-1.

Polymerase chain reaction

PCR was implemented using previously described conditions (Mahieux *et al.,* 1997a, 1997b). Briefly, high-molecular-weight DNA was extracted from PBMC samples before culture by a classic phenol–chloroform technique. Each reaction mixture contained 1.5 μg of DNA, 0.2 mM deoxynucleoside triphosphate mix (Boehringer-Mannheim, Mannheim, Germany), 10 μl of a 10$\times$ reaction buffer (Perkin-Elmer Cetus, Gaithersburg, MD), 25 mM concentration each of oligonucleotide primers (Pharmacia, Uppsala, Sweden), and 2.5 U of *Taq* DNA polymerase (Perkin-Elmer Cetus) in a total volume of 100 μl. For each sample, HTLV-1- or HTLV-2-specific primers and appropriate internal oligonucleotide probes were used. For all PCR analyses, amplification mixes were made in a special room physically separated from the laboratory, and during all steps, positive displacement pipettes were used to prevent PCR contamination. For all the PCR experiments, after denaturation at 94°C for 5′, the reaction mixtures containing DNA were cycled 35 times (*gag, pol, env, tax*) or 40 times (LTR) at 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min. An extension of 2 min per cycle was realized with an extension of 10 min on the last cycle.

STLV-1 LTR amplification

A semi-nested PCR was performed as previously described (Mahieux *et al.,* 1997a). The first fragment (8255–LTRU5E) of 467 bp (8266–8733) was amplified, and 2 μl of this initial PCR were used for the second run, generating a fragment (8255–420LTR) of 433 bp (8266–8699). The second fragment (3PLTR–5PLTR) of 778 bp (1–778)

was amplified, and 2 μl of the initial PCR were used for the second PCR run, amplifying an inner fragment (4PLTR–5PLTR) of 657 bp (122–778).

STLV-1 *gag* amplification

A single PCR was done with, as previously described (Kawase *et al.,* 1992), Gag3–1301 and Gag4–1420 HTLV-1-specific primers and the specific probe Gag 1380. The amplified fragment was 120 bp.

STLV-1 *pol* amplification

Two different PCRs were done using either SK110 and SK111 primers and the HTLV-1-specific probe SK112 or SK58 and SK59 HTLV-2-specific primers with the HTLV-2-specific SK60 probe as previously described (Kwok *et al.,* 1988). The fragments were 185 and 102 bp, respectively.

STLV-1 *env* amplification

A semi-nested PCR was done using Env1 and Env22 as outer primers and Env1 and Env2 as inner primers as previously described (Mahieux *et al.,* 1997a). The probe used was PE-12. The amplified fragment was 569 bp long.

STLV-1 *tax* amplification

A simple PCR was done using kkpx1 and kkpx2 primers and the probe SK43 as previously described (Mahieux *et al.,* 1997b). The amplified fragment was 203 bp long.

Cloning and sequencing

PCR products of STLV-1 *env* and LTR were purified on 1.5% agarose gels (Geneclean, Bio101, La Jolla, CA) cloned in the PCR Bluescript(SK)$^+$ vector (Stratagene, La Jolla, CA). One clone (*env*) or two overlapping clones (LTR) were sequenced for each sample (Sequenase, version 2.0; U.S. Biochemicals, Beverly, MA) as previously described (Mahieux *et al.,* 1997a, 1997b). The *pol* products (1 PCR reaction) (185-bp fragment SK110–SK111) were directly sequenced from the PCR products using the Applied Biosystems model 373 automatic DNA sequencer. The 12 nucleotide sequences reported here have been submitted to the GenBank nucleotide sequence database and have been assigned the accession numbers AF035542–AF03545 (*env*), AF035538–AF035541 (LTR,) and AF038426–AF038429 (pol).

Phylogenetic analyses

Genetic variations of the newly described baboon sequences were compared with sequences representing previously described strains of human and simian viruses. Three data sets were examined separately and were composed of either 44 complete LTR, 65 partial LTR, or 93 *env* gene sequences amplified by PCR. Sequences were compiled and aligned using the algorithm of Needleman and Wunsch (1970) of the program GCG version 8 (Genetics Computer Group, 1996) and verified visually. Computations of nucleotide frequencies, estimation of the transition-to-transversion ratio, and numbers of variable sites among sequences were performed using MEGA version 1.01 (Kumar *et al.,* 1993) Phylogenetic analysis of LTR and *env* sequences used two algorithms: MP and minimum evolution estimated by NJ. Because these two methods use different optimality criteria, concordance between the resultant topologies was viewed as evidence of the true phylogeny. Phylogenetic reconstruction with maximum parsimony was performed by the program PAUP* (with permission from D. Swofford). A heuristic search with the 44 complete LTR and the 65 partial LTR weighted transitions 5:1 relative to transversions and included stepwise addition for starting trees, simple addition of sequences, and branch swapping performed by tree–bisection–reconnection, and with the option of collapsed branch, length was zero. Gaps were treated as a fifth character state. Maximal parsimony search conditions of the 93 *env* gene sequences used similar options except branch swapping was changed to the nearest-neighbor interchange and transitions were weighted 2:1 with transversions. Neighbor-joining analysis of LTR sequences used the Tajima-Nei (1984) model of substitution performed by PAUP*. Heuristic search conditions used a starting tree obtained by NJ with tree–bisection–reconnection for branch swapping. Analysis of the *env* sequences used the Kimura two-parameter model of substitution with PAUP*. Bootstrap resampling was used in conjunction with both MP and distance-based methods to test the reliability of the data in phylogenetic analysis. Bootstrap analysis consisted of 100 iterations with identical search parameters as stated above. Within each iteration using PAUP*, the maximum number of trees saved was set as 100. Bootstrap proportions of $>$70% were considered strong support for the adjacent node (Hillis and Bull, 1993).

ACKNOWLEDGMENTS

We thank Dr. Diane Agay, Dr. Antonia Van Uye, and Dr. Philippe Michel for access to the troop of *P. anubis* and *P. ursinus* of the Centre de Recherches du Service de Santé des Armées (CRSSA, La Tronche, France). We are grateful to L. Edgard, F. Sarrauton, and H. Chaussard for collecting the blood samples from the monkeys and to Mrs. M. Van Beveren for technical assistance during the serological experiments This study was supported by Agence Nationale de Recherches sur le SIDA (ANRS). R.M. was a fellow from the CANAM and the Virus Cancer Prévention Association. G. M. was a fellow from the Délégation Générale au Réseau International des Instituts Pasteur et Instituts Associés (Prof. J.L. Durosoir).

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