Simian T-Cell Lymphotropic Virus Type 1 from *Mandrillus sphinx* as a Simian Counterpart of Human T-Cell Lymphotropic Virus Type 1 Subtype D

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A recent serological and molecular survey of a semifree-ranging colony of mandrills (*Mandrillus sphinx*) living in Gabon, central Africa, indicated that 6 of 102 animals, all males, were infected with simian T-cell lymphotropic virus type 1 (STLV-1). These animals naturally live in the same forest area as do human inhabitants (mostly Pygmies) who are infected by the recently described human T-cell lymphotropic virus type 1 (HTLV-1) subtype D. We therefore investigated whether these mandrills were infected with an STLV-1 related to HTLV-1 subtype D. Nucleotide and/or amino acid sequence analyses of complete or partial long terminal repeat (LTR), *env*, and *rex* regions showed that HTLV-1 subtype D-specific mutations were found in three of four STLV-1-infected mandrills, while the remaining monkey was infected by a different STLV-1 subtype. Phylogenetic studies conducted on the LTR as well as on the *env* gp21 region showed that these three new STLV-1 strains from mandrills fall in the same monophyletic clade, supported by high bootstrap values, as do the sequences of HTLV-1 subtype D. These data show, for the first time, the presence of the same subtype of primate T-cell lymphotropic virus type 1 in humans and wild-caught monkeys originating from the same geographical area. This strongly supports the hypothesis that mandrills are the natural reservoir of HTLV-1 subtype D, although the possibility that another monkey species living in the same area could be the original reservoir of both human and mandrill viruses cannot be excluded. Due to the quasi-identity of both human and monkey viruses, interspecies transmission episodes leading to such a clade may have occurred recently.

The primate T-cell lymphotropic/leukemia viruses type 1 (PTLV-1) found in humans (HTLV-1) and simian primates (STLV-1) are closely related retroviruses, having epidemiological and molecular properties in common (7). However, their origin and evolutionary relationship, as well as their modes of dissemination, are still unclear and a matter of discussion (7, 8, 13, 16, 27, 29, 32, 33). Most of these viruses cannot be separated into distinct phylogenetic lineages according to species of origin. Their phylogenetic intermixing has been interpreted as evidence for past and recent interspecies transmission episodes (13, 16, 17, 24–27, 32, 33). However, with regard to the viral transmission from monkeys to humans, this hypothesis is supported by a limited number of observations. The first evidence was brought about by the strong clustering of three STLV-1 strains isolated from chimpanzees with HTLV-1 subtype B strains obtained either from inhabitants of Zaire or Pygmies from the Central African Republic and Cameroon (13, 17, 29). Despite the unknown origins in the wild of most of these captive chimpanzees, and the fact that some had been inoculated with HTLV-1 strains of unknown subtype (29), the low divergence among the HTLV-1 and STLV-1 strains (on average, fewer than 2% of nucleotide substitutions in a 522-bp portion of the *env* gene) was interpreted as resulting from interspecies transmission episodes (13, 17, 29). The HTLV-1 subtype C strains which correspond to Australo-Melanesian viruses also have a common ancestor with simian viruses, probably originating from macaques (*Macaca tonkeana*) (11). In this case, an average genetic divergence of 12% among the human and simian viruses suggested a longer time (probably several millennia) of in vivo-separated evolution in remote human populations after the initial interspecies transmission episodes (11). Finally, although more than 30 STLV-1 strains from Africa have been partially characterized, none of them was reported to be interspersed within the strains of the two other HTLV-1 molecular subtypes (A and D) that have been described previously (17). Subtype A, also named Cosmopolitan because it is the most dispersed subtype worldwide, is endemic in west Africa, where it was possibly disseminated through migrations of infected human populations (7, 8). Subtype D, recently described by our group, is rare and seems to be restricted to central African inhabitants, mainly Pygmies (1, 17, 21). The goal of this study was to search for a simian counterpart of HTLV-1 subtype D.

In 1997, blood samples were obtained from four STLV-1-infected male mandrills (*Mandrillus sphinx*) living in a semifree-ranging colony of the Centre International de Recherches Médicales (CIRMF) in Gabon, central Africa. This colony was founded in 1980 with 14 wild-caught mandrills originating mostly in the forest area of southern Gabon. Two of them tested STLV-1 seropositive on their arrival (5). Mandrill Mnd9, studied in the present report, was one of the original colony members. The remaining three mandrills (Mnd13, Mnd15, and Mnd18) seroconverted in 1990, 1993, and 1995, respectively.
FIG. 1. Sequence alignment of 79 amino acids corresponding to a fragment of the p27/rex protein obtained for two African HTLV-1 subtype D variants (H2-3 and 230101), five mandrill STLV-1 strains (Mnd7, Mnd9, Mnd13, Mnd15, and Mnd18), and representative strains of all other known HTLV-1 subtypes, i.e., subtypes A (strain ATK), B (strain EL), and C (strain Mels).

respectively, and probably acquired the virus through oral exposure (biting for male dominance) to another STLV-1-infected original member, mandrill Mnd7, which was dead at the onset of this study (23). None of these animals have ever been injected with any simian or human blood materials. They have not been living with any other monkey species, thus preventing possible episodes of interspecies transmission. Serological and molecular studies were performed to confirm the presence of HTLV-1/STLV-1 infection in the four animals. Specific HTLV-1/STLV-1 antibodies were detected by an indirect immunofluorescence assay using either HTLV-1-producing cells (MT2) or HTLV-2-producing cells (C19). In all four cases, the antibody titers were higher in MT2 than in C19 cells. Furthermore, the four mandrills demonstrated a typical HTLV-1 Western blot (HTLV2-3; Diagnostic Biotechnology) profile, with a stronger reactivity against p19 than against p24 and reactivities against both the recombinant gp21 and the specific HTLV-1 gp46 peptide MTA-1 (data not shown). DNAs extracted from uncultured peripheral blood mononuclear cells from the four mandrills were tested by six PCR experiments with HTLV-1- and/or HTLV-2-specific long terminal repeat (LTR), gag, pol, env, and tax primers and probes using PCR conditions as described previously (17, 18). Proviral sequences related to HTLV-1, but not HTLV-2, were detected in the four animals with all the primers (with the exception of SK110/111, which gave negative results for the DNA extracted from the peripheral blood mononuclear cells Mnd15), confirming data obtained previously on a small fragment of the rex region for three of these animals (23).

To examine the evolutionary relationships between the STLV-1 strains present in the mandrills (strains Mnd9, Mnd13, Mnd15, and Mnd18 [named after the corresponding mandrills]) and the different available HTLV-1 subtype D strains, we performed comparative analyses of a 238-bp (nucleotides 7341 to 7579 of the ATK prototype) fragment of the rex gene, a 522-bp fragment of the env gene (nucleotides 6065 to 6589 of ATK), and either a 418-bp fragment (nucleotides 360 to 778 of ATK) or the complete LTR (755 bp). The rex gene fragments from the STLV-1 strains from mandrills (except that from Mnd18) were already available in the GenBank database under accession no. Z71190 to Z71224 (23). All other fragments were amplified by using previously described primers, probes, and PCR conditions (17, 18). The HTLV-1 and STLV-1 rex fragments were amplified at different times in two different laboratories (one at the CIRMF in Gabon and one in France) by researchers working entirely independently. The env and LTR regions were amplified by different investigators and cloned in different vectors and at different times (HTLV-1 env and LTR regions in 1995–1996 and STLV-1 env and LTR regions in 1997). To prevent contamination, the PCR steps were done by different investigators using positive displacement pipettes. The STLV-1 env, rex, and LTR PCR products were purified on 1.5% agarose gels and cloned in the PCR script SK vector that had never been used for HTLV-1 subtype D cloning (Strategene, La Jolla, Calif.). DNA from recombinant clones was then extracted, purified (Midiprep; Qiagen, Milden, Germany), and manually sequenced (Sequenase, version 2.0; U.S. Biochemicals, Beverly, Mass.).

The rex gene fragments from strains Mnd13 and Mnd15 had an identical sequence and 99% sequence identity with Mnd7 and 99.2% with Mnd18, while the sequence of Mnd9 diverged from them by 2.5% (4). The corresponding rex gene fragments from two HTLV-1 subtype D viruses, H2-3 and 230101, both from two male Pygmies from Cameroon, were sequenced. One virus (H2-3) was described in a previous work (17), and the other (230101) was recently detected during an epidemiological study performed among Pygmy populations living in the rain forest area of southwestern Cameroon (19). H2-3 and 230101 nucleotide sequences exhibited 99.6% similarity to each other. They were compared with the 238-bp rex sequences from STLV-1 Mnd7, Mnd9, Mnd13, Mnd15, and Mnd18 and with reference sequences of HTLV-1 subtype A (ATK), B (EL), and C (Mels). H2-3 and 230101 exhibited 99.6 and 99.15% sequence similarity, respectively, with Mnd7; 100 and 99.6%, respectively, with Mnd7; 100 and 99.6%, respectively, with Mnd7; 100 and 99.6%, respectively, with Mnd18; and 98.8 and 97%, respectively, with Mnd9. Moreover, H2-3 and 230101 showed only 97.5 and 97.9% nucleotide sequence similarity, respectively, with ATK; 96.6 and 97%, respectively, with EL; and 95.4 and 95.8%, respectively, with Mels. For further analysis of the rex gene fragment, the amino acid sequences of STLV-1 Mnd7, Mnd9, Mnd13, Mnd15, and Mnd18 and of HTLV-1 H2-3 and 230101 were compared to amino acid reference sequences of all HTLV-1 subtypes (Fig. 1). Compared to the subtype A (ATK and TSP-1) and B (EL) sequences, sequences from STLV-1 Mnd7, Mnd13, Mnd15, and Mnd18 showed five amino acid substitutions while that from Mnd9 possessed none of them. The H2-3 sequence presented the five same substitutions, while strain 230101 possessed four of them. The association of these five amino acid substitutions was determined to be subtype D specific and has never been reported for any other HTLV-1 or STLV-1 subtypes. Moreover, another substitution (Q→P) at position 4 [Fig. 1] was present in both HTLV-1 subtype D viruses as well as in two mandrill STLV-1 strains (Mnd7 and Mnd18). This result confirmed that the STLV-1 strains Mnd7, Mnd13, Mnd15, and Mnd18 were closely related to the HTLV-1 subtype D strains while strain Mnd9 corresponds to a different molecular subtype.
To further confirm that some STLV-1 strains from mandrills were the simian counterpart of HTLV-1 subtype D strains, we searched, in a 522-bp fragment of the STLV-1 env gene, for a combination of four nucleotide mutations which were recently shown to be HTLV-1 subtype D specific (17). Three sequences, of strains Mnd13, Mnd15, and Mnd18, showed these four specific mutations (A\_C at position 343, G\_A at position 394, C\_T at position 461, and C\_T at position 520), while the env sequence of Mnd9 showed a mutation only at position 461 (data not shown).

The complete LTR sequence (755 bp) was then characterized for strains Mnd9 and Mnd13, while only a partial 418-bp sequence was obtained for Mnd15 and Mnd18. Sequence comparison was performed on the largest common sequence (705 bp) available for Mnd9 and Mnd13, the LTR sequences of two HTLV-1 subtype D LTR sequences available (H2-3, Pyg19, and CMR229), obtained with the CLUSTAL W program and minimal manual editing. Arrows indicate HTLV-1–STLV-1 subtype D-specific mutations. Dots indicate sequence identity with HTLV-1 strain ATK.

Phylogenetic analyses were performed on sequences from

FIG. 2. LTR nucleotide sequence alignment of two complete (Mnd9 and Mnd13) and two partial (Mnd15 and Mnd18) STLV-1 strains from mandrills, two other prototypes of African STLV-1 strains (STLVAG and STLVCH), two HTLV-1 subtype B strains from Pygmies (H24 and 12503), and all three partial or complete HTLV-1 subtype D LTR sequences available (H2-3, Pyg19, and CMR229), obtained with the CLUSTAL W program and minimal manual editing. Arrows indicate HTLV-1–STLV-1 subtype D-specific mutations. Dots indicate sequence identity with HTLV-1 strain ATK.
the new mandrill STLV-1 strains and all other HTLV-1 and STLV-1 strain sequences available from the database. One tree was generated on a comparison of the 522-bp fragment of the gp21 \textit{env} gene sequences for the four mandrill strains (Mnd9, Mnd13, Mnd15, and Mnd18), 39 other STLV-1 strains, 93 HTLV-1 strains, and 1 HTLV-2 strain (MO), which was used as the root for the tree display. It is important to note that this portion of the \textit{env} gene is the region of the provirus for which the greatest number of sequences is available. The second tree was built on a sequence comparison of the complete LTRs of two mandrill STLV-1 strains, Mnd9 and Mnd13, 7 other STLV-1 strains, and 39 HTLV-1 strains. The divergent sequence of STLV-1 PTM3 was used as the root for the LTR tree. As shown in Fig. 2, strain Mnd15 and Mnd18 LTR sequences were very similar to the Mnd13 LTR sequence (99.75 and 99.52% identities, respectively). We therefore did not sequence the complete LTRs of these two strains, which were for this reason not included in the LTR phylogenetic analysis. Alignments were performed by using the program Sequin, version 2.45 (12). The 522-bp-long alignment of the gp21 \textit{env} gene sequences showed no gap. The 774-bp-long alignment of LTR sequences was modified by hand to correct the position of the gaps. Both phylogenetic analyses were performed by using the software PHYLIP, version 3.5 (2). The trees were built from a distance matrix (function DNADIST) by using the neighbor-joining (NJ) method (function NEIGHBOR). This method uses a transition/transversion ratio of 2. The robustness of the NJ trees was assessed by bootstrap resampling of the multiple alignment. Bootstrap values were obtained from 500 data sets, randomly resampled from the original alignments (functions SEQBOOT and CONSENSE) (10). Since tree-building algorithms rely on different assumptions, we used either one (\textit{env} analysis) or two (LTR analysis) other different methods (DNA maximum parsimony [DNApars] and maximum-likelihood [ML]) to increase the reliability of the derived tree topologies. These trees were created by using the functions DNAML and DNAPARS in the PHYLIP program. Because most of the HTLV-1 and STLV-1 sequences available in the databases comprise either the \textit{env} or LTR region, it was not possible to conduct analyses with the same data sets.

In the phylogenetic analyses performed on the \textit{env} gene (Fig. 3), the HTLV-1 subtypes A, B, C, and D were identified on the basis of consistent topological association in the two phylogenetic methods used. Although subtypes A and B were divergent and confined within subtypes, these two clusters were supported by low bootstrap values for NJ (50 and 42%, respectively) and for DNApars (37 and 18%, respectively). These values, which were reported previously (17), could be explained by the low number of informative subtype-specific mutations in this \textit{env} fragment. Interestingly, the sequence of STLV-1 PPAS5X28, which has previously been described as the STLV-1 sequence closest to the HTLV-1 subtype A, was now gathered together with the Mnd9 strain sequence as well as with four recently published STLV-1 strain sequences from different African monkey species, mainly baboons (strains SuF1, PH6356, 1621, and SM) (3, 14, 28, 30). However, as recently reported (14), this STLV-1 group was not well supported by bootstrap values (10% for NJ and 22% for DNApars). The divergent Australo-Melanesian HTLV-1 subtype C and Asian STLV-1 strain sequences formed highly supported phylogenetically clades in both analyses. The HTLV-1 subtype D was well discriminated and associated with
high bootstrap values (77% for NJ and 65% for DNApars). The high bootstrap value previously reported for HTLV-1 subtype D (77% for NJ) (17) remained unchanged, despite the addition of the three sequences for STLV-1 Mnd13, Mnd15, and Mnd18, supporting the hypothesis of a monophyletic HTLV-1–STLV-1 subtype D.

Phylogenetic analyses conducted on complete LTR sequences by three different methods (NJ, DNApars, and ML) led to similar configurations of a tree with the four distinct and phylogenetically highly supported HTLV-1 genomic subtypes A, B, C, and D (Fig. 4). The HTLV-1 subtype A, including the reference Japanese and west African strains ATK and HS35, was well defined, with bootstrap values of 94% for NJ and of 86% for DNApars and a $P < 0.01$ for ML. The central African HTLV-1 subtype B was also well defined with bootstrap values of 75% for NJ and 54% for DNApars and a $P < 0.01$ for ML. The third group of PTLV-1 strain sequences, clearly separated from the other clades, corresponds to distant Asian HTLV-1 (subtype C) and STLV-1 strains. The HTLV-1 subtype D was previously described with a bootstrap value of 99% for NJ (17).

In conclusion, we report here the first evidence of a close molecular and phylogenetic relationship between the strains of the HTLV-1 subtype D and three STLV-1 strains from mandrills most likely derived from a single wild-caught mandrill. The molecular subtype D now includes five HTLV-1 strains, the three mandrill STLV-1 strains (Mnd13, Mnd15, and Mnd18) from Gabon described herein, and probably Mnd7. Of the five HTLV-1 strains, three (H2-3, CMR229, and 230101) were obtained from Pygmies living in southwestern Cameroon, one (Pyg19) was from another Pygmy living in the southwestern part of the Central African Republic, and one (2318) was from a non-Pygmy healthy carrier living in southern Gabon. Therefore, all PTLV-1 subtype D carriers appear to live in a restricted geographical territory of the western part of central Africa. Contacts between inhabitants of this region and mandrills are not rare and may account for recurring episodes of interspecies viral transmission. As a possibility, blood-blood contacts may have occurred during the hunting of mandrills, whose meat is highly appreciated in these areas. Furthermore, young mandrills, like several other monkey species, are also frequently kept as household pets in villages of these regions (6).

The striking observation that only males were infected by this virus subtype in the mandrill colony has been interpreted as the result of preferred male-to-male transmission resulting from fights, with bloody injuries, for male dominance within the colony and rare sexual transmissions (23). In the few well-studied colonies of monkeys infected with other STLV-1 subtypes (Macaca fuscata, Macaca tonkeana, and Cercopithecus aethiops), infection occurred more frequently in females than in males. This phenomenon was also observed for human groups infected with HTLV-1 subtype A, B, or C (15, 19, 20, 22, 34). Interestingly, all five HTLV-1 subtype D carriers reported so far are males, and four of them are Pygmies and hunters and therefore have high risks of acquiring simian viruses by blood contacts. The different HTLV-1 subtype D-infected individuals are not related and are living in widely separated areas of the western part of central Africa (17, 19,
21). Therefore, they have no possibility of any sexual contacts among themselves. Furthermore, in contrast to the other HTLV-1 subtypes, there is not yet evidence of sexual transmission of HTLV-1 subtype D. A study of three HTLV-1 subtype D-infected Pygmy families has been performed (17, 19). In one family, the wife of the HTLV-1 subtype D-infected index patient was HTLV-1 seronegative, and the wives in the two other families were infected by HTLV-1 subtype B strains. The hypothesis of a low interhuman transmission rate of HTLV-1 subtype D might be due to a low proviral load, as suggested by preliminary studies, and may account for the apparent low prevalence observed, even for the remote Pygmy populations (21). This must, however, be confirmed by further epidemiological studies.

Although the time of divergence between HTLV-1 and STLV-1 is extremely difficult to estimate and the rate of mutation of both viruses is unknown, viral divergence between humans and simians may be inferred from the sequence similarity and clustering. We report herein the first case of STLV-1 viruses, derived from a wild-caught mandrill, shown to be genetically indistinguishable from HTLV-1 subtype D viruses. These results may suggest recent cross-transmission episodes. No comparable conclusion can yet be reached for any other HTLV-1 and STLV-1 groups. However, one might also consider an alternative hypothesis—that another monkey species is the natural reservoir of both mandrill and human type D viruses.

Finally, extensive sequence variation between PTLV strains obtained from wild-caught monkeys of the same species has been reported previously. For instance, Papio cynocephalus as well as P. hamadryas, which are naturally infected by African STLV-1 subtypes, were also found to be infected with Macaca mulatta STLV-1 strains (13, 31). In the present study, mandrills were shown to be the natural carriers of two different viral molecular subtypes, one being related to subtype B of HTLV-1 and the other being the simian counterpart of HTLV-1 subtype D.

Nucleotide sequence accession numbers. The four env sequences, four LTR sequences, and one rex sequence for STLV-1 have been registered under GenBank accession no. AF045928 to AF045931 (env), AF045932 to AF045935 (LTR), and AF082875 (Mnd18 rex).

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REFERENCES

![Phylogenetic tree generated by the NJ method with the complete LTR (755 bp long in the HTLV-1 strain ATK reference sequence) of sequences from two mandrill STLV-1 strains, Mnd9 and Mnd13, 7 other STLV-1 sequences, and 39 HTLV-1 sequences recovered from the databases. The numbers at some nodes or branches (bootstrap values; in italics) indicate frequencies of occurrence for 500 trees. The bar represents 0.01 nucleotide substitution per site or 1% divergence.](http://jvi.asm.org/Downloaded_from http://jvl.asm.org)


