Cross-species transmission of retroviruses is common in Cameroon. To determine risk for simian T-cell lymphotropic virus (STLV) transmission from nonhuman primates to hunters, we examined 170 hunter-collected dried blood spots (DBS) from 12 species for STLV. PCR with generic and group-specific long terminal repeat primers showed that 12 (7%) specimens from 4 nonhuman primate species were infected with STLV. Phylogenetic analyses showed broad diversity of STLV, including novel STLV-1 and STLV-3 sequences and a highly divergent STLV-3 subtype found in *Cercopithecus mona* and *C. nictitans* monkeys. Screening of peripheral blood mononuclear cell DNA from 63 HTLV-seroreactive, PCR-negative hunters did not identify human infections with this divergent STLV-3. Therefore, hunter-collected DBS can effectively capture STLV diversity at the point where pathogen spillover occurs. Broad screening using this relatively easy collection strategy has potential for large-scale monitoring of retrovirus cross-species transmission among highly exposed human populations.

**Primate T-Lymphotrophic Viruses (PTLVs)** are composed of simian and human T-lymphotropic viruses (STLVs and HTLVs, respectively). To date, only 4 major PTLV groups have been identified. PTLV-1, PTLV-2, and PTLV-3 include human (HTLV-1, HTLV-2, and HTLV-3) and simian (STLV-1, STLV-2, and STLV-3) viruses (1–6). PTLV-4 consists of only HTLV-4, which was recently reported in a person in Cameroon known to have been exposed to nonhuman primates (NHPs) (7). A simian counterpart of this virus has yet to be identified. More recently, a highly divergent STLV-1–like virus from captive macaques (*Macaca arctoides*) has been described (8); further analysis suggests a possible new lineage outside the diversity of PTLV-1, provisionally named STLV-5 (9).

Both HTLV-1 and HTLV-2 have spread globally and are pathogenic in humans (10–13). HTLV-1 causes adult T-cell leukemia/lymphoma, HTLV-1–associated myelopathy/tropical spastic paraparesis, and other inflammatory diseases in <5% of those infected (2,11,13). HTLV-2 is less pathogenic than HTLV-1 but has been associated with a neurologic disease similar to HTLV-1–associated myelopathy/tropical spastic paraparesis (10,12). HTLV-1 and HTLV-2 are known to be transmitted by sexual contact, breast-feeding, and exposure to contaminated blood or blood products through transfusion and injection drug use (11–13). Less is known about the transmissibility and pathogenicity of HTLV-3 and HTLV-4. Nevertheless, recent full-length sequence analysis of the HTLV-3 (14,15) and HTLV-4 genomes (W.M. Switzer et al., unpub. data) suggested ancient origins of these viruses and showed functional motifs that affect viral expression and possibly oncogenesis (14,15; W.M. Switzer et al., unpub. data).

The recent discovery of HTLV-3 and HTLV-4 demonstrates that the diversity of PTLV is far from understood...
(7). Studies have shown that the diversity of HTLV is directly related to the genetic diversity of the STLVs from which the primary zoonotic infection originated (5,16). Every HTLV-1 subtype except A is composed of genetically related HTLV-1 and STLV-1 strains from many different primate species, all found geographically near each other. Similarly, PTLV-3s exhibit broad diversity among NHPs in the wild; currently, 3 subtypes have been suggested according to the geographic origin of the strains (17): East African STLV-3 subtype A includes STLV-3 (PH969) found in a baboon (Papio hamadryas) from Eritrea (18) and from captive gelada baboons (Theropithecus gelada) (19); West and Central African STLV-3 subtype B includes STLV-3 (CT-604) and STLV-3 (CT-602) found among mangabeys (Cercocebus torquatus) from Cameroon (20) and STLV-3 (PPAF3) from baboons (P. hamadryas papio) from Senegal (17); and Central African STLV-3 subtype C includes divergent strains (Cni217 and Cni227) from Cercocebus nictitans monkeys from Cameroon (21). Together, this clustering by geography rather than host species suggests the ease with which STLVs are transmitted among NHPs and possibly to humans (2,3,5,22,23).

We used a hunter-based field surveillance approach to investigate STLV diversity among primate bushmeat samples collected from 12 NHP species in different locations in Cameroon. We also sampled NHPs in the surrounding region for the STLV source of the HTLV-4–infected individual. In addition, we examined the utility of using dried blood spots (DBS) in the field for surveillance of cross-species transmission of retroviruses.

Materials and Methods

Sample Collection and Preparation

Before the study began, Institutional Animal Care and Use Committee approvals were obtained. Self-identified hunters from 4 study sites in southern Cameroon volunteered to collect DBS from freshly hunted NHP bushmeat (Figure 1). Hunters were educated about the risks associated with direct contact with NHPs and about appropriate prevention measures. Preliminary identification of hunted species was undertaken by using pictographs of NHPs common in the region (24). Confirmation of species was performed by analysis of mitochondrial cytochrome oxidase subunit II and/or glucose-6-phosphate dehydrogenase sequences (25,26). Over 2 years, a total of 362 DBS from hunted NHPs were collected on Whatman filter paper (Kent, UK), air dried, and stored locally at room temperature in envelopes with desiccant until processed. Nucleic acids were extracted by using the NucliSens nucleic acid isolation kits (bioMérieux, Durham, NC, USA). DNA quality and yield were determined by semiquantitative PCR amplification of the β-actin gene as previously described (27,28). DNA preparation and PCR assays were performed in different laboratories specifically outfitted for processing and testing of NHP samples only, according to established precautions to prevent contamination. Specimens were coded by using a strategy previously described (15).

PTLV Sequence Detection and Sequence Analysis

DNA samples from NHPs were tested for tax sequences by using generic and nested PCR assays capable of detecting viruses from all 4 major PTLV groups (7,19,27). Phylogenetic resolution was achieved by analysis of long terminal repeat (LTR) sequences using PTLV group-specific primers (7). PCR amplification of overlapping regions of the 5′ and 3′ STLV-1 LTR (4) and partial STLV-3 LTR (7,19) sequences were performed using primers and conditions reported elsewhere. A PCR-based genome-walking approach (15) was used to obtain partial viral genome fragments of a highly divergent PTLV from monkeys Cmo8699AB and Cni7867AB (Table 1). (NHPs are coded as follows: the first letter of the genus is followed by the first 2 letters of the species name, e.g., Cag, Cercocebus agilis; Cni, C. nictitans; Cmo, C. mona; and Lal, Lophocebus albigena. The last 2 letters in the code indicate the study site, e.g., AB, Abat; MV, Mvangan.)

To screen humans for the divergent STLV-3 subtype, we developed a nested PCR assay based on STLV-3 (Cmo8699AB) tax sequences. Similar strategies have been used to screen for the novel HTLV-3 and HTLV-4 viruses in NHP hunters from Cameroon (1,7). DNA for PCR test-
reactive blood donor DNA samples (data not shown), which could reliably detect 10 copies of STLV-3 (Cmo8699AB). New STLV-3 (Cmo8699AB) sequences were not amplified by using QIAquick PCR extraction, and 170 (79%) of the 215 yielded adequate amplifiable DNA (Table 2). Blood clots and limited volumes of blood on some DBS accounted for poor DNA yield of some samples.

Because of the limited amount of DBS material available, we used a PCR assay that detects sequences from all 4 major PTLV groups. We observed a broad range of PTLV diversity over a wide geographic distribution. Of the 170 samples screened, 12 (7%) from 4 NHP species were positive for PTLV sequences. The last 2 letters in the code indicate the study site: AB, Abet.

Table 1. Nucleotide sequences of primer sets used to amplify tax and long terminal repeat sequences of simian T-lymphotropic virus–3 (Cmo8699AB and Cni7867AB)*

| Region | Primer set | Forward primer and sequence (5’ → 3’) | Reverse primer and sequence (5’ → 3’) | bp |
|-------|------------|---------------------------------------|---------------------------------------|----|
| tax   | Outer      | GTACCCGTCTCAGTCTTCGCCGAT              | GAIGAYGIATACACAGATGCTG               | 779|
|       | Inner      | TTACTGGCCACCTGGCTCAGCAC             | TIGGGYAGGICCGGAAATCAT               | 658|
|       | Outer      | CCCTCAAGTCCTCCAACCGCCGC          | TACCGCAGCGTCATGGAGGTG               | 244|
|       | Inner      | AAGTTCTCCTCCTCCTCCTCATT             | TGGTAGAGTTAAGCAGACAGTGGT             | 174|
| tax-LTR | Outer    | CATCCGGACCAACTAGGGCGCTT          | TCCGTACCGYCTYYRCCTTTTATAG       | 721|
|       | Inner      | AAAAAAATCCCCAACAAAGCCTT                   | TCCGTACCGYCTYYRCCTTTTATAG       | 695|
|       | Inner      | CAGCCCAACCGGCAGACAGAATT                | TCCGTACCGYCTYYRCCTTTTATAG       | 589|
| LTR   | Outer      | CTCTGACGTCTCTCCTGCTCTTGT           | ATCCCGGAGGAGCCCCCA                | 612|
|       | Inner      | CCAGGAAAACCTTTAACCACCCA              | ATCCCGGAGGAGCCCCCA                | 585|

*bp, basepair; LTR, long terminal repeat; I, inosine; S, G/C; Y, T/C; R, A/G. Nonhuman primates are coded as follows: the first letter of the genus is followed by the first 2 letters of the species name: Cmo, Cercopithecus mona; Cni, C. nictitans. The last 2 letters in the code indicate the study site: AB, Abet.
†Primers used to screen human peripheral blood mononuclear cell DNA for simian T-lymphotropic virus–3 (Cmo8699AB)–like tax sequences.
‡Primer set used for Cni7867AB.
§Primer set used for Cmo8699AB.

Results

A total of 362 DBS representing 12 NHP and prosimian species were collected (Figure 1), of which 215 (60%) were of adequate quality and quantity for nucleic acid extraction, and 170 (79%) of the 215 yielded adequate amenable DNA (Table 2). Blood clots and limited volumes of blood on some DBS accounted for poor DNA yield of some samples.

Because of the limited amount of DBS material available, we used a PCR assay that detects sequences from all 4 major PTLV groups. We observed a broad range of PTLV diversity over a wide geographic distribution. Of the 170 samples screened, 12 (7%) from 4 NHP species were positive for PTLV tax sequences (Table 3). Phylogenetic analysis of the short tax sequences from these 12 samples showed that 7 NHPs (2 Cercopithecus agilis and 5 C. nictitans monkeys) were infected with STLV-1 and that 3 (C. agilis, C. nictitans, and Lophocebus albigena monkeys) were infected with STLV-3 (Figure 2, Table 3). We did not find any evidence of STLV-2, HTLV-4–like STLV, or dual STLV-1 and STLV-3 infections as have been found in C. agilis monkeys in other studies (25).

Samples Cmo8699AB and Cni7867AB, each collected near the same village but from 2 different NHP species, contained nearly identical STLV sequences with highest nucleotide identity to viruses in the PTLV-3 group, but they exhibited high divergence in this small region of tax (Figure 2, EU152279, EU152280–EU152281, and EU152282–EU152293, respectively.)
between viral subtypes in the genus is generally <3% within viral subtypes and up to 15% between viral subtypes in the tax region (7), the 7% divergence seen in the tax sequences of STLV-3 (Cmo8699AB) and STLV-3 (Cni7867AB), along with the clustering of these viruses outside the diversity of other STLV-3–like viruses (9,21), suggested the identification of a new and highly divergent PTLV-3 subtype (Figure 3; Table 4).

**Phylogenetic Resolution of a Novel PTLV-3 Subtype**

The identification of highly divergent STLV-3–like sequences in Cmo8699AB and Cni7867AB was investigated further by additional analyses of a larger tax sequence (1,015 bp). Both tax sequences were nearly identical (99.9%) despite nucleic acid extraction, PCR amplification, and sequencing for both animals all being performed on different days. Analysis of mitochondrial DNA sequences also confirmed the Cercopithecus species of each monkey and the absence of admixtures of specimens from different NHP species. STLV-3 (Cmo8699AB) tax sequences share 72%–74% nucleotide identity with PTLV-1, PTLV-2, and PTLV-4, but they have the highest nucleotide identity to the PTLV-3 group (82%–84%) in this highly conserved

| Taxonomic name (common name) | DBS extracted, no. | ß-actin positive, no. (%) | tax positive, no. (%) | STLV-1 LTR positive, no. | STLV-3 LTR positive, no. |
|-----------------------------|-------------------|-------------------------|-----------------------|-------------------------|-------------------------|
| Old World monkeys          |                   |                         |                       |                         |                         |
| Cercocebus agilis (agile mangabey) | 6               | 3 (50)                  | 3 (100)               | 2                       | 1                       |
| C. cephus (moustached monkey) | 41              | 32 (78)                 | 0                     | 0                       | 0                       |
| C. mona (mona monkey)       | 40               | 36 (90)                 | 1 (2.7)               | 0                       | 1                       |
| C. neglectus (de Brazza's monkey) | 1               | 1 (100)                 | 0                     | 0                       | 0                       |
| C. nictitans (spot-nosed monkey) | 98             | 73 (74.5)               | 7 (9.6)               | 4                       | 2                       |
| C. pogonias (crowned monkey) | 9                | 8 (88.8)                | 0                     | 0                       | 0                       |
| Colobus guereza (guereza colobus) | 3               | 2 (66.7)                | 0                     | 0                       | 0                       |
| Lophocebus albigena (gray-cheeked monkey) | 10              | 9 (90)                  | 1 (11.1)              | 0                       | 1                       |
| Prosimian                   |                   |                         |                       |                         |                         |
| Arctocebus aureus (golden angwantibo) | 2              | 1 (50)                  | 0                     | 0                       | 0                       |
| A. calabarensis (calabar angwantibo) | 2              | 2 (100)                 | 0                     | 0                       | 0                       |
| Galago alleni (Allen’s galago) | 1               | 1 (100)                 | 0                     | 0                       | 0                       |
| Perodicticus potto (potto)  | 2                | 2 (100)                 | 0                     | 0                       | 0                       |
| Total                       | 215              | 170 (79.1)              | 12 (7.1)              | 6 (3.5)                 | 5 (2.9)                 |

*DBS, dried blood spots; STLV, simian T-lymphotropic virus; LTR, long terminal repeat.
†Samples negative for ß-actin sequences were not tested for primate T-lymphotropic virus sequences.

Table 3. Primate T-lymphotropic virus diversity and geographic distribution among wild nonhuman primates, Cameroon*

| No. | Code    | Species (common name) | Site     | Province   | PTLV (subtype) |
|-----|---------|-----------------------|----------|------------|----------------|
| 1   | Cag9812NL | Cercopithecus agilis (agile mangabey) | Ngoila   | East       | STLV-1 (f)     |
| 2   | Cag9813NL | C. agilis             | Ngoila   | East       | STLV-1 (f)     |
| 3   | Cag9748NL | C. agilis             | Ngoila   | East       | STLV-3 (b)    |
| 4   | Cmo8699AB | C. mona (mona monkey) | Abat     | Southwest  | STLV-3 (d)     |
| 5   | Cni10026NL | C. nictitans (spot-nosed monkey) | Ngoila   | East       | STLV-1†       |
| 6   | Cni10225NL | C. nictitans         | Ngoila   | East       | STLV-1 (d)    |
| 7   | Cni8284NY  | C. nictitans         | Nyabissan| South      | STLV-1 (d)    |
| 8   | Cni8286NY  | C. nictitans         | Nyabissan| South      | STLV-1 (d)    |
| 9   | Cni8348NY  | C. nictitans         | Nyabissan| South      | STLV-1 (d)    |
| 10  | Cni7882AB | C. nictitans         | Abat     | Southwest  | STLV-3 (d)    |
| 11  | Cni7867AB | C. nictitans         | Abat     | Southwest  | STLV-3 (d)    |
| 12  | Lal9589NL | Lophocebus albigena (gray-cheeked monkey) | Ngoila   | East       | STLV-3 (b)    |

*PTLV, primate T-lymphotropic virus; STLV, simian T-lymphotropic virus. Nonhuman primates are coded as follows: the first letter of the genus is followed by the first 2 letters of the species name: Cag, C. agilis; Cmo, C. mona; Cni, C. nictitans; Lal, L. albigena. The last 2 letters in the code indicate the study site: AB, Abat; NL, Ngoila; NY, Nyabissan.
†Subtype not determined.
Simian T-Lymphotropic Virus Diversity, Cameroon

Figure 2. Primate T-lymphotropic virus (PTLV) phylogeny inferred by using 161-bp tax sequences. New sequences from nonhuman primates (NHPs) from Cameroon in this study are in boldface. Support for the branching order was determined by 1,000 bootstrap replicates; only values ≥60% are shown. Branch lengths are proportional to the evolutionary distance (scale bar) between the taxa. Nonhuman primates are coded as follows: the first letter of the genus is followed by the first 2 letters of the species name: Cmo, Cercocebus mona; Cni, Cercocebus nictitans; Cto, Cercocebus torquatus; Ppa, Papio papio; Ph, Papio hamadryas; Tge, Theropithecus gelada. Cag, Cercocebus agilis; Lal, Lophocebus albigena; Mnd and msp, Mandrillus sphinx; PanP and PP, Pan paniscus; Pha, Pan troglodytes; Ggo, Gorilla gorilla; Tan, tamarind monkey; Cag, Cercocebus agilis; Mar, Macaca arctoides; Pha, Papio hamadryas; Pan, Papio anubis; Bab, baboon; HYB, hybrid baboon (Pha X Pan); Cae, Chlorocebus aethiops (AGM, African green monkey); Cpo, Cercocebus pogonias; Cmi, Cercocebus mitis; Cce, Cercocebus cephus; Ang, Allenopithecus nigroviridis; Wrc, Western red colobus. The last 2 letters in the code indicate the study site: AB, Abat; MV, Mvangan; NY, Nyabissan; NL, Ngoila; MN, Manyemen; BA, Bangourain; MA, Massangam; YI, Yingui; ND, Ndikinimike; NG, Ngovayan; SA, Sobia; LE, Lomie; MO, Mouloundou.

region where intragroup sequence identity is typically >90%. Phylogenetic analysis of 881-bp tax sequences (Figure 4) from these 2 monkeys with other PTLVs, using bovine leukemia virus as an outgroup, inferred a new lineage with high bootstrap support (99%) from the diversity of other PTLV-3 subtypes (larger tax sequences representing PTLV-3 subtype C were not available for inclusion in this analysis), which suggests a long, independent evolution of this divergent virus.

Similar PTLV-3 tree topologies were obtained by analysis of 275-bp LTR sequences (Figure 5) in which STLV-3 (Cmo8699AB) and STLV-3 (Cni7867AB) had only 70%–74% identity to LTRs from other members of the PTLV-3 group that share >84% nucleotide identity between subtypes A and B (data not shown). LTR sequences from other STLV-3-infected C. agilis and C. nictitans monkeys from Cameroon reported elsewhere were not available from GenBank (9, 21, 25) and thus were not included in the current phylogenetic analysis. Combined, the phylogenetic analyses of the tax (Figures 3, 4) and LTR (Figure 5) sequences show that STLV-3 (Cmo8699AB) and STLV-3 (Cni7867AB) each form a distinct cluster with high bootstrap support from the other known STLV-3 subtypes. On the basis of nomenclature proposed by others (17), our results suggest that these viruses are members of a novel PTLV-3 subtype that we tentatively name as STLV-3 West African subtype D.

Origin of STLV-3 (Cmo8699AB)

To estimate the divergence times of the most recent common ancestor of STLV-3 (Cmo8699AB), we performed additional molecular analyses. We found that the molecular clock hypothesis was not rejected for the 881-bp alignment of PTLV and bovine leukemia virus tax sequences in both PAUP* (http://paup.csit.fsu.edu) and Tree-Puzzle (www.tree-puzzle.de) analyses (p = 0.012 and 0.858, respectively), which is consistent with results obtained recently by others (29). Using a molecular clock model and a tree calibration date estimated for the origin of Melanesian HTLV-1 ≈40,000–60,000 years ago (15, 19, 29, 30), we inferred the evolutionary rate for PTLV to be 9.17 × 10⁻⁷ to 1.38 × 10⁻⁶ substitutions/site/year, which is consistent with rates determined previously both with and without a molecular clock model of evolution (15, 17, 20, 29–31). The evolutionary rate for STLV-3 (Cmo8699AB) is estimated to be 2.11 × 10⁻⁷ to 3.16 × 10⁻⁶, and the most common recent ancestor is inferred to have occurred ≈92,072–138,560 years ago, which suggests an ancient origin and perhaps the identification of one of the oldest viruses in the PTLV-3 group.

Broad STLV-3 Diversity in Wild NHPs

Sequence analysis of the STLV-3 LTR sequences from Cni7882AB, Cag9748NL, and Lal9589NL showed that all
Table 4. High genetic diversity of novel STLV-3 (subtype D) tax sequences compared to prototypical PTLV-3s*

| Nonhuman primate | Subtype D | Subtype C | Subtype B | Subtype A |
|------------------|-----------|-----------|-----------|-----------|
|                  | Cmo8699AB† | Cni217‡   | Cni3034   | 2026ND    |
|                  | 99.9      | 99.5      | 100.0     | 93.1      |
|                  | Cni7867AB†| 93.2      | 98.8      | 93.5      |
|                  | Cni227‡   | 93.5      | 99.1      | 93.1      |
|                  | Cni3038   | 93.5      | 99.0      | 83.7      |
|                  | Cto604†   | 99.8      | 92.4      | 93.5      |
|                  | CtoNG409  |          | 93.2      | 93.7      |
|                  | PPAF3     |          | 97.5      | 90.8      |
|                  | Ph969     |          | 97.5      | 90.8      |
|                  | Tge2117   |          | 97.5      | 90.8      |

*STLVs, simian T-lymphotropic viruses; PTLVs, primate T-lymphotropic viruses. Boldface indicates intersubgroup identities; shading indicates intrasubgroup identities. Nonhuman primates are coded as follows: the first letter of the genus is followed by the first 2 letters of the species name: Cmo, Cercopithecus mona; Cni, C. nictitans; Cto, Cercopithecus torquatus; Ppa, Papio papio; Ph, Papio hamadryas; Tge, Theropithecus gelada. The last 2 letters in the code indicate the study site: AB, Abat; ND, Ndikimikeri.

†Partial tax sequence (1015 bp).
‡Partial tax sequence (219 bp).
§Partial tax sequence (170 bp).
¶Partial tax sequence (202 bp).

Figure 3. Identification of a novel primate T-lymphotropic virus (PTLV-3) subtype by phylogenetic inference of 202-bp tax sequences with PTLV prototypes and partial sequences from 3 Cercopithecus nictitans (Cni217, Cni227, and Cni3038) reported elsewhere (9,21) and those identified in the current study (in boldface). GenBank accession numbers for the previously reported partial simian T-lymphotropic virus (STLV–3) tax sequences included in this analysis are AY039033, AF412120, and AM746647–AM746673. Support for the branching order was determined by 1,000 bootstrap replicates; only values ≥60% are shown. Branch lengths are proportional to the evolutionary distance (scale bar) between the taxa. See Figure 2 legend for abbreviations.

were infected with distinct STLV-3s. LTR sequences (283 bp) from animal Cag9748NL shared the greatest identity (≥97%) with those from HTLV-3 (Pyl43) and STLV-3 (Cto604) from a red-capped mangabey from Cameroon (1,20). The 282-bp LTR sequence from Cni7882AB shared the highest nucleotide identity (99%) to STLV-3 (CtoNG409), a red-capped mangabey from neighboring Nigeria (31). The phylogeographic clustering of these sequences supports further the proposed subtype classification of STLV-3 by geographic origin rather than by host species (17,19,20,25,31). In contrast, the 432-bp LTR sequence from L. albigena mangabeys (Lal9589NL) was more divergent; it shared only 10%–16% nucleotide identity with all PTLV-3 LTR sequences. Similar to the phylogenetic relationships inferred with the small PTLV-1–like tax sequences, the LTR sequence from L. albigena mangabeys (Lal9589NL) formed a new lineage within the diversity of other PTLV-3 sequences from west-central Africa (Figure 5). Although these results need to be confirmed with additional LTR sequences from this virus and from other STLV-3–infected L. albigena mangabeys (9), our findings demonstrate a host range and geographic distribution of STLV-3 that is more widespread than previously considered.

Phylogenetic Analysis of STLV-1 Diversity

To investigate further the genetic relationships inferred with the small PTLV-1–like tax sequences, we obtained LTR sequences for 6 of 7 PTLV-1–positive samples by using established primer-pair combinations (3,4,7). Phylogenetic analysis of these sequences, including those identified from our study of infected NHP hunters in Cameroon (7), showed that 4 sequences from C. nictitans mon-
keys all clustered in the central African HTLV-1 subtype D clade, consisting of STLV-1 from Mandrillus sphinx and Cercopithecus pogonias monkeys and HTLV-1 sequences from Cameroon (Figure 6). The STLV-1 (Cni10225NL) LTR sequence was phylogenetically closest to the HTLV-1 (1842LE) strain from an NHP hunter from Cameroon (Figure 6). Similarly, LTR sequences from 2 C. agilis (Cag9812NL and Cag9813NL) monkeys clustered within the HTLV-1 clade (Figure 6). Combined, these results support further the primate origin of the HTLV-1D and -1F subtypes. We were unable to amplify STLV-1 LTR sequences from DBS samples from a C. nictitans monkey (Cni10026NL) that was positive for STLV-1 tax sequences, possibly because of low viral load in this animal, lower sensitivity of the LTR primers, or genetic variances at the LTR primer binding sites. The absence of STLV-1 LTR sequences in this monkey is not likely to have resulted from infection with an STLV-1/STLV-3 recombinant after dual infection of animal Cni10026NL with both viruses because samples from this animal were repeatedly negative for STLV-3 tax and LTR sequences.

Absence of Novel STLV-3 Subtype Sequences in NHP Hunters

Given the prevalence of the STLV-3 subtype D virus in at least 2 monkey species in Cameroon, we investigated whether this new subtype was also present among NHP hunters in Cameroon. Peripheral blood mononuclear cell DNA samples were available from a previous study of 63 NHP hunters who had a wide range of WB seroreactivity to HTLV (7). HTLV sequences were not previously detected in the DNA of these persons when either generic or groupspecific primers were used (7). All 63 NHP hunters were also negative for STLV-3 (Cmo8699AB) tax-specific sequences, which suggests the absence of this virus in this subset of persons with broad WB seroreactivity to HTLV.

Discussion

Widespread exposure to a broad range of NHP body fluids and tissues encountered during hunting, butchering, or keeping primates as pets has been implicated in the emergence of 3 different retrovirus genera: HIV, HTLV, and, more recently, simian foamy virus (2–5,7,16,28,32). Although little is known about the public health implications of simian foamy virus infection, the social, medical, political, and economic consequences of HIV and HTLV global spread and pathogenicity after cross-species transmission are enormous. The recent discovery of HTLV-3 and HTLV-4 in NHP hunters from Cameroon doubles the number of known deltaretroviruses in humans (7). This
same study also identified novel STLV-1–like infections in NHP hunters (7). These discoveries demonstrate that the diversity of PTLV is far from understood and that zoonotic infection with STLV continues in persons exposed to NHPs (7). Thus, understanding the diversity, prevalence, and geographic range of STLV infection in areas where frequent contact with wild NHPs is common provides useful information about the origin and emergence of HTLV and the risks for exposure to these and possibly other simian viruses.

We demonstrated that monkeys from 3 distant locations in the rain forests of southern Cameroon are infected with a broad range of highly diverse STLV. Our detection of a 7% prevalence of STLV infection among hunted wild monkeys is comparable to the 8%–11% seroreactivity to PTLV recently found in monkey and ape samples collected mostly at urban bushmeat markets in Cameroon (9,25). Through analysis of LTR and larger tax sequences from C. mona and C. nictitans monkeys in our study, we have identified new divergent STLV-3–like strains that form a unique PTLV-3 clade that we designated subtype D. Altogether, these results extend further the range of PTLV diversity and suggest a founder effect for PTLV evolutionary radiation in this region where most PTLV groups have been identified.

Given the propensity of STLV to cross species boundaries, the increased frequency of hunting and demand for primate bushmeat in Africa, and the apparent broad diversity of STLV subtypes in Cameroon (9,21), it is tempting to speculate that human infection with this unique STLV-3 subtype will or may have already occurred. However, PCR testing of DNA samples from Cameroon NHP hunters with broad HTLV WB patterns showed no evidence of STLV-3 (Cmo8699AB)–like infections. Possible explanations for this negative finding include the testing of only a limited number of available samples, an unknown sensitivity for serologic detection of this virus with assays used in our study (7), an unknown prevalence and host range of this virus in NHPs, and other factors such as low transmissibility to humans. Nevertheless, the discovery of this novel PTLV-3 subtype in 2 monkey species and an apparent ancient origin of this lineage suggest a possible wider distribution of this variant. Therefore, the ease with which STLVs can cross species barriers and potentially be transmitted during NHP-hunting practices warrants increased surveillance for human infection with this divergent subtype. A similar strategy involving intensified screening of NHP hunters was successful in the discovery of HTLV-3 (1,7) and HTLV-4 (7).

Finding a broad range of STLVs in simian DBS indicates that persons exposed to NHPs from Cameroon are at increased risk for infection with highly diverse STLV. Indeed, phylogenetic analysis of PTLV-1 LTR sequences shows that the new STLV-1 from C. nictitans monkeys identified in the current study is most similar to HTLV-1 from Cameroon NHP hunters (7). Similarly, the clustering of STLV-1 from C. agilis monkeys with LTR sequences obtained from a person from Liberia provides additional support for the primate origin of the HTLV-1F clade (33). Combined, these findings further support the hypothesis of active cross-species transmission of STLV to humans in this region (7).

Moreover, we show that use of DBS collected in the field in collaboration with hunters provides a good tool for surveillance of emerging retroviral infections at the NHP-hunter interface. Convenient and cost-effective, this collection strategy provides a unique opportunity to examine zoonotic transmission at the point where pathogen spillover occurs. In conjunction with longitudinal sampling of hunters, these collections have the potential to enable simultaneous documentation of both sides of a cross-species transmission event.
In summary, we found broad diversity of STLV in NHPs from Cameroon and identified a novel STLV-3 subtype. These results provide increasing evidence that the diversity and geographic distribution of PTLVs are much greater than previously thought. Bushmeat hunting, an ancient and common practice in many parts of Africa, is an ideal interface for cross-species transmission of retroviruses between NHPs and humans. Contact with body fluids and blood during hunting and butchering of NHP bushmeat exposes humans to a plethora of simian retroviruses, as demonstrated here and elsewhere (7,23,25,32,34,35), and increases the likelihood of emerging diseases in humans. To predict and possibly prevent the next retrovirus pandemic, expanded surveillance is needed for these and other retroviruses in their natural host reservoirs and in persons exposed to NHPs (7,36,37).

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References

1. Calattini S, Chevalier SA, Duprez R, Bassot S, Froment A, Mahieux R, et al. Discovery of a new human T-cell lymphotropic virus (HTLV-3) in Central Africa. Retrovirology. 2005;2:30. DOI: 10.1186/1742-4690-2-30
2. Gessain A, Mahieux R. Epidemiology, origin and genetic diversity of HTLV-1 retrovirus and STLV-I simian affiliated retrovirus [in French]. Bull Soc Pathol Exot. 2000;93:163–71.
3. Mahieux R, Chappey C, Georges-Courbot MC, Dubreuil G, Maulepe R, Georges A, et al. Simian T-cell lymphotropic virus type 1 from Mandrillus sphinx as a simian counterpart of human T-cell lymphotropic virus type 1 subtype D. J Virol. 1998;72:10316–22.
4. Meertens L, Rigoulet J, Maulepe C, Van Beveren M, Chen GM, Diop O, et al. Molecular and phylogenetic analyses of 16 novel simian T-cell leukemia virus type 1 from Africa: close relationship of STLV-1 from Alenopithecus nigeroviridis to HTLV-1 subtype B strains. Virology. 2001;287:275–85. DOI: 10.1006/viro.2001.1018
5. Slattery JP, Franchini G, Gessain A. Genome evolution, patterns of global dissemination, and interspecies transmission of human and simian T-cell leukemia/lymphotropic viruses. Genome Res. 1999;9:525–40.
6. Van Brussel M, Salem M, Liu HF, Goubau P, Desmyter J, Vandamme AM. The discovery of two new divergent STLVs has implications for the evolution and epidemiology of HTLVs. Rev Med Virol. 1999;9:155–70. DOI: 10.1002/(SICI)1099-1654 (199907/09)9:3<155::AID-RMV242>3.0.CO;2-3
7. Wolfe ND, Heneine W, Carr JK, Garcia AD, Shannugam V, Tamooufe U, et al. Emergence of unique primate T-cell lymphotropic viruses among central African bushmeat hunters. Proc Natl Acad Sci U S A. 2005;102:7994–9. DOI: 10.1073/pnas.0507134102
8. Van Dooren S, Meertens L, Lemey P, Gessain A, Vandamme AM. Full-genome analysis of a highly divergent simian T-cell lymphotropic virus type 1 strain in Macaca arctoides. J Gen Virol. 2005;86:1953–9. DOI: 10.1099/vir.0.80520-0
9. Liégeois F, Lafay B, Switzer WM, Locatelli S, Mpoudi-Ngole E, Loul S, et al. Identification and molecular characterization of new STLV-1 and STLV-3 strains in wild-caught nonhuman primates in Cameroon. J Gen Virol. 2008;89:405–17. DOI: 10.1099/vir.0.006230-0
10. Araujo A, Hall WW. Human T-lymphotropic virus type II and neurological disease. Ann Neurol. 2004;56:10–9. DOI: 10.1002/ana.20126
11. Pirozzi FA, Carneiro-Pirozzi AB, Catalan-Soares BC, Murphy EL. Global epidemiology of HTLV-I infection and associated diseases. Oncogene. 2005;24:6058–68. DOI: 10.1038/sj.annonc.1208968
12. Roucoux DF, Murphy EL. The epidemiology and disease outcomes of human T-lymphotropic virus type II. AIDS Rev. 2004;6:144–54.
13. Yamashita M, Ido E, Miura T, Hayami M. Molecular epidemiology of HTLV-I in the world. J Acquir Immune Defic Syndr Hum Retrovirology. 1996;13(Suppl 1):S124–31. DOI: 10.1097/00042560-199600001-00021
14. Calattini S, Chevalier SA, Dوقع R, Afonso P, Fromant A, Gessain A, et al. Human T-cell lymphotropic virus type 3: complete nucleotide sequence and characterization of the human tax3 protein. J Virol. 2006;80:9876–88. DOI: 10.1128/JVI.00799-06
15. Switzer WM, Qari SH, Wolfe ND, Burke DS, Folks TM, Heneine W. Ancient origin and molecular features of the novel human T-lymphotropic virus type 3 revealed by complete genome analysis. J Virol. 2006;80:7427–38. DOI: 10.1128/JVI.00690-06
16. Korallnik UF, Boeri E, Saxinger WC, Monico AL, Fullen J, Gessain A, et al. Phylogenetic associations of human and simian T-cell leukemia/lymphotropic virus type I strains: evidence for interspecies transmission. J Virol. 1994;68:2693–707.
17. Meertens L, Gessain A. Divergent simian T-cell lymphotropic virus type 3 (STLV-3) in wild-caught *Papio hamadryas papio* from Senegal: widespread distribution of STLV-3 in Africa. J Virol. 2003;77:782–9. DOI: 10.1128/JVI.77.1.782-789.2003

18. Goubau P, Van Brussel M, Vandenheede AM, Liu HF, Desmyter J. A primate T-lymphotropic virus, PTLV-L, different from human T-lymphotropic viruses types I and II, in a wild-caught baboon (*Papio hamadryas*). Proc Natl Acad Sci U SA. 1994;91:2848–52. DOI: 10.1073/pnas.91.7.2848

19. Van Dooren S, Shanmugam V, Bhullar V, Parekh B, Vandenheede AM, Heneine W, et al. Identification in gelada baboons (*Theropithecus gelada*) of a distinct simian T-cell lymphotropic virus type 3 with a broad range of Western blot reactivity. J Gen Virol. 2004;85:507–19. DOI: 10.1099/vir.0.19630-0

20. Meertens L, Mahieux R, Mauclere P, Lewis J, Gessain A. Complete sequence of a novel highly divergent simian T-cell lymphotropic virus from wild-caught red-capped mangabeys (*Cercopithecus torquatus*) from Cameroon: a new primate T-lymphotropic virus type 3 subtype. J Virol. 2002;76:259–68. DOI: 10.1128/JVI.76.1.259-268.2002

21. Van Dooren S, Salemi M, Pourrut X, Peeters M, Delaporte E, Van Ranst M, et al. Evidence for a second simian T-cell lymphotropic virus type 3 in *Cercopithecus nictitans* from Cameroon. J Virol. 2001;75:11939–41. DOI: 10.1128/JVI.75.23.11939-11941.2001

22. Song KJ, Nerurkar VR, Saitou N, Lazo A, Blakeslee JR, Miyoshi I, et al. Genetic analysis and molecular phylogeny of simian T-cell lymphotropic virus type I: evidence for independent virus evolution in Asia and Africa. Virology. 1994;199:56–66. DOI: 10.1006/viro.1994.1097

23. Van Dooren S, Verschoor EJ, Fagrouch Z, Vandenheede AM. Phylogeny of primate T lymphotropic virus type 1 (PTLV-1) including various new Asian and African non-human primate strains. Infect Genet Evol. 2007;7:374–81. DOI: 10.1016/j.meegid.2006.04.005

24. Kingdon J. The Kingdon field guide to African mammals. London: Academic Press; 1997.

25. Courgnaud V, Van Dooren S, Liegeois F, Pourrut X, Abela B, Loul S, et al. Simian T-cell leukemia virus (STLV) infection in wild primate populations in Cameroon: evidence for dual STLV type 1 and type 3 infection in agile mangabeys (*Cercopithecus agilis*). J Virol. 2004;78:4700–9. DOI: 10.1128/JVI.78.9.4700-4709.2004

26. Switzer WM, Salemi M, Shanmugam V, Gao F, Cong ME, Kuiken C, et al. Ancient co-speciation of simian foamy viruses and primates. Nature. 2005;434:376–80. DOI: 10.1038/nature03341

27. Busch MP, Switzer WM, Murphy EL, Thomson R, Heneine W. Absence of evidence of infection with divergent primate T-lymphotropic viruses in United States blood donors who have seroin-determinate HTLV test results. Transfusion. 2000;40:443–9. DOI: 10.1046/j.1537-2995.2000.40040443.x

28. Wolfe ND, Switzer WM, Carr JK, Bhullar VB, Shanmugam V, Tumouf E, et al. Naturally acquired simian retrovirus infections in central African hunters. Lancet. 2004;363:932–7. DOI: 10.1016/S0140-6736(04)67587-5

29. Lemey P, Pybus OG, Van Dooren S, Vandamme AM. A Bayesian statistical analysis of human T-cell lymphotropic virus evolutionary rates. Infect Genet Evol. 2005;5:291–8. DOI: 10.1016/j.meegid.2004.04.005

30. Salemi M, Desmyter J, Vandamme AM. Tempo and mode of human and simian T-lymphotropic virus (HTLV/STLV) evolution revealed by analyses of full-genome sequences. Mol Biol Evol. 2000;17:374–86.

31. Meertens L, Shanmugam V, Gessain A, Beer BE, Tooze Z, Heneine W, et al. A novel, divergent simian T-cell lymphotropic virus type 3 in a wild-caught red-capped mangabey (*Cercopithecus torquatus torquatus*) from Nigeria. J Gen Virol. 2003;84:2723–7. DOI: 10.1099/vir.0.19253-0

32. Hahn BH, Shaw GM, De Cock KM, Sharp PM. AIDS as a zoonosis: scientific and public health implications. Science. 2000;287:607–14. DOI: 10.1126/science.287.5453.607

33. Salemi M, Van Dooren S, Audenaert E, Delaporte E, Goubau P, Desmyter J, et al. Two new human T-lymphotropic virus type I phylogenetic subtypes in seroindeterminates, a Mbuti pygmy and a Gabonese, have closest relatives among African STLV-I strains. Virology. 1998;246:277–87. DOI: 10.1006/viro.1998.9215

34. Aghokeng AF, Liu W, Bibollet-Ruche F, Loul S, Mpoudi-Ngole E, Laurent C, et al. Widely varying SIV prevalence rates in naturally infected primate species from Cameroon. Virology. 2006;345:174–89. DOI: 10.1016/j.virol.2005.09.046

35. Peeters M, Courgnaud V, Abela B, Auzel P, Pourrut X, Bibollet-Ruche F, et al. Risk to human health from a plethora of simian immunodeficiency viruses in primate bushmeat. Emerg Infect Dis. 2002;8:451–7.

36. Wolfe ND, Dunavan CP, Diamond J. Origins of major human infectious diseases. Nature. 2007;447:279–83. DOI: 10.1038/nature05775

37. Wolfe ND, Switzer WM, Heneine W. Emergence of novel retroviruses. Washington: American Society for Microbiology Press; 2006.

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