Origin of HTLV-1 in Hunters of Nonhuman Primates in Central Africa

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Of 78 Gabonese individuals who had received bites from nonhuman primates (NHPs) while hunting, 7 were infected with human T lymphotropic virus (HTLV-1). Five had been bitten by gorillas and were infected with subtype B strains; however, a 12-year-old girl who was severely bitten by a Cercopithecus nictitans was infected with a subtype D strain that was closely related to the simian T lymphotropic virus (STLV-1) that infects this monkey species. Her mother was infected with a subtype B strain. These data confirm that hunters in Africa can be infected by HTLV-1 that is closely related to the strains circulating among local NHP game. Our findings strongly suggest that a severe bite represent a risk factor for STLV-1 acquisition.

Keywords. STLV-1; HTLV-1; interspecies transmission; human infection; Gabon; wild-born nonhuman primate.

Widespread distribution of different types of human immunodeficiency virus (HIV) and human T lymphotropic virus (HTLV) in humans from their transmission through well-known routes, including mother-to-child transmission, sexual intercourse, and contact with contaminated blood products. Identification of closely related retroviruses (simian immunodeficiency virus [SIV] and simian T lymphotropic virus [STLV]) among nonhuman primates (NHPs), especially in West and Central Africa, strongly indicates a simian origin for types of HIV and HTLV. It has been suggested that simian retroviruses can occasionally be acquired by humans through direct exposure to the body fluids of infected NHPs during hunting or butchering bush-meat or contact with pets [1–5]. The exact mechanisms and modes of acquisition of SIVs and STLVs by humans from noncaptive animals are poorly understood and a matter of discussion [1–5]. We therefore studied hunters in 2 countries of central Africa (Gabon and Cameroon), who are considered to be at high risk for possible interspecies transmission of retroviruses because of frequent contact with NHP body fluids. We first studied simian foamy viruses and demonstrated that they are relatively easily transmitted to humans through severe bites, mainly from apes and to a lesser extent from small monkeys [6–8]. Our next goal was to search for evidence of STLV-1 transmission from NHPs to humans.

MATERIAL AND METHODS

As reported earlier [7], blood was collected from 78 individuals in southeastern Gabon (10 women, 59 men, and 9 children with a mean age of 46 years) who had received severe bites or scratches from NHPs while hunting or playing with pets. Blood was not collected from villagers living in the same area who had not been exposed to NHP. Most of the bites were from apes (mostly gorillas) and some from small monkeys. The monkey or ape species was determined at interviews with use of pictures of different NHPs. Ethical approval was received from the Gabonese Ethical Committee (Research authorization no. 00093/ MSP/SG/SGAQM). The aim of the study was explained orally to all participants, and individual written consent was obtained for blood sampling; parents’ written consent was obtained for participating children. Aliquots of plasma and buffy coat were stored at −20°C until the end of the field mission and then transferred on dry ice to the Centre International de Recherches Médicales de Franceville, where they were kept at −80°C until analysis.

For wide serological screening for HTLV-1, all plasma samples were tested with 2 enzyme-linked immunosorbent assays: Platelia HTLV-I New (Bio-Rad, France) and Vironostika HTLV-1/2 (Bio-Mérieux, France). Plasma samples that were positive or borderline in 1 or both assays were then tested by Western blot (HTLV Blot 2.4, MP Diagnostics Suisse, Switzerland). All samples that were positive or indeterminate for HTLV in this test were also analyzed in another confirmatory test (INNO-LIA HTLV I/II Score; Immunogenetics, Gent, Belgium) [9].
For molecular studies, DNA was extracted from 200 µL of buffy coat with the QIAamp DNA mini kit (Qiagen, Courtaboeuf, France) according to the manufacturer’s recommendations. HTLV proviral load was measured in a multiplex real-time polymerase chain reaction (PCR) assay involving molecular beacon probes that allow simultaneous detection, differentiation, and quantification of HTLV types 1, 2, and 3, as described elsewhere [10]. For sequence analysis, PCR amplifications were performed with HTLV-1- or HTLV-2-specific primers located within the long terminal repeat (LTR) and envelope glycoprotein (Env) coding regions, as described elsewhere [11]. For phylogenetic analysis, the Env (522 bp) and LTR (479 bp) sequences were aligned with the ClustalX program and then analyzed manually with the editor program of the MEGA package [12]. Phylogenetic relations were reconstructed by the distance neighbor-joining method in the Kimura 2-parameter model [13], and confidence intervals were estimated for 1000 replicates with the MEGA package.

For cell culture, peripheral blood mononuclear cells (PBMC) were separated from blood containing EDTA with a Ficoll-Paque gradient and were then washed 3 times with phosphate-buffered saline. After washing, the PBMC were suspended in Roswell Park Memorial Institute (RPMI) 1640 growth medium supplemented with 20% heat-inactivated foetal bovine serum (Gibco BRL, Eragny, France), a 1% penicillin–streptomycin mixture, 1% L-glutamine 200 mmol/L, and 20 U/mL of human recombinant interleukin 2 (Roche Diagnostics, Mannheim, Germany). The lymphocytes were stimulated with 3 µg/mL of concanavalin-A (Sigma-Aldrich, Saint Quentin Fallavier, France) and then incubated at 37°C in 5% CO2. To maintain the cells, 90% of medium was changed twice a week.

RESULTS AND DISCUSSION

Seven of the 78 studied individuals were infected with HTLV-1, as demonstrated by serological and/or PCR analyses. Of these, 5 had been bitten by a gorilla, 1 by a mandrill, and 1 by a small monkey (Cercopithecus nictitans; Supplementary Table 1). The last case was a 12-year-old girl (Gab-16H) living in a remote village of the Haut-Ougoué region of Gabon, who had been bitten on the right hand by a C. nictitans 5 years before our visit while hunting monkeys with her father. The bite was quite severe, with a clearly visible scar (Figure 1A). For further epidemiological and molecular investigations, blood samples were also obtained from the girl’s parents and brothers as well as from 85 individuals living in the same village. Her mother was infected...
Figure 2. Phylogenetic tree of HTLV-1/STLV-1 LTR constructed with a 450-bp portion by the neighbor-joining method with HTLV-1/STLV-1 strains, including the new strains from people bitten by nonhuman primates and people living in the same region of Gabon (in red). In subtype D, the blue cluster contains case Gab-16H (accession No. GQ344419) and all known Cercopithecus nictitans LTR sequences; MKB51 sequence (GQ344420) from a blood sample obtained from Gab-16H 1 year after first identification; MKB51CC sequence (GQ344421) obtained after 1 year of PBMC culture. Within subtype B, MKB43 (GQ344433) is the LTR sequence obtained directly from the PBMC and after 1 year of PBMC culture (MKB43CC, GQ344434) from the mother of Gab-16H. The numbers along ancestral segments indicate the robustness of each node, as estimated by 1000 bootstrap samplings of the data. The Genbank accession numbers of the new sequences in the phylogenetic tree are GQ344413—GQ344445. Abbreviations: HTLV, human T lymphotropic virus; LTR, long terminal repeat; PBMC, peripheral blood mononuclear cells; STLV, simian T lymphotropic virus.
with HTLV-1, but her father and brothers were not (Figure 1B). Of the 85 villagers (none of whom reported a bite from a NHP), 22 (7 men and 15 women; mean age, 66 years) were infected with HTLV-1. Western blot results showed that the pattern of the HTLV in the girl was different from that of her mother and of the other infected villagers, with faint seroreactivity mainly against p24, p19, and GD21 but not MTA-1 (Figure 1C), considered to be HTLV positive according to the manufacturer and confirmed by the INNO-LIA assay (Figure 1C). In contrast, plasma from the girl’s mother and from the other 22 infected villagers showed complete HTLV-1 seroreactivity by Western blot, suggesting either that the girl had a low HTLV-1 proviral load or that she was infected with a slightly different virus. Another possibility was that HTLV-1 titer conversion was still in progress. To discriminate between these 2 hypotheses, proviral load was measured in the PBMC of the infected girl, and blood samples were collected 3 times over a 2-year period. As seen in the supplementary data presented in Supplementary Table 1, the HTLV proviral load in the girl’s PBMC was low; however, a low proviral load was also found in 2 hunters (Gab-1H and Gab-59H). Three consecutive Western blots performed over 2 years confirmed the low reactivity and showed no significant variation over time (Figure 1C). This suggests that the strain with which Gab-16H was infected was different from the HTLV-1 found typically in central African populations.

Molecular analyses based on LTR (Figure 2) and Env (gp21; Supplementary Figure 1) gene sequences were performed on Gab-16H samples obtained twice at a 2-year interval and from PBMC after 1 year of culture in vitro. Blast comparative analyses confirmed that the girl was infected with a rare HTLV-1 subtype D strain [14], whereas only typical subtype B strains were found in the other infected villagers tested, including the girl’s mother and the 6 hunters bitten by NHPs (Figure 2). The Gab-16H strain had close nucleotide identity (99.6% homology for the LTR region and 98.4% homology for the gp21 Env gene) with STLV-1 sequences (CN01-gab accession numbers: GQ344422 and GQ344455, respectively) found previously in an STLV-1-infected C. nictitans in the same region of Gabon [15].

This conclusion was confirmed by the phylogenetic analyses, which clearly positioned the strain obtained at various times from the girl (16H, MKB51, and MKB51CC) within the subtype D clade, with a bootstrap value of 82% for the LTR fragment (Figure 2) and of 89% for the 522 bp of the Env gene (Supplementary Figure 1).

Altogether, our findings strongly suggest that the girl was infected by STLV-1 through contact with a C. nictitans. First, the severe bite on the girl’s hand would have resulted in contact between her blood and the saliva, and perhaps the blood of the monkey. Second, the girl’s PBMC contained an STLV-1/HTLV-1 subtype D strain nearly identical to known C. nictitans strains, including 1 from the same area of Gabon. Third, all the other villagers, including the girl’s mother, were infected with HTLV-1 subtype B. The young age of the girl argues against sexual acquisition of this virus, and subtype D is moreover quite rare in inhabitants of this area [11].

It is difficult to determine the exact origin of the other HTLV-1 subtype B strains found in the villagers (including the girl’s mother), which could have been zoonotic transmission through bites and/or butchering or human-to-human spread, and in the hunters that had been bitten by gorillas. In this central African region, apes are infected by strains of the same STLV-1/HTLV-1 B subtype. Nevertheless, our data confirm that hunters in Africa can be infected by HTLV-1 strains that are closely related to those circulating among local NHP game, as has been shown in Côte d’Ivoire and Cameroon [4, 5]. Our work extends these findings to Gabon.

In conclusion, our data strongly suggest that a severe bite can be a risk factor for acquisition of STLV-1. Nevertheless, more precise epidemiological studies are required to define the main risk factors associated with zoonotic acquisition of HTLV-1 subtype B strains in NHP hunters and others bitten or scratched by NHPs. The frequency of transmission, which may vary according to the NHP species, raises the possible role of molecular determinants (eg, restriction factors) or behavioral factors (eg, different degrees of NHP aggressiveness) in this zoonotic event [4]. Finally, the increase in consumption of bush-meat and the high HIV endemicity in this area imply that more people with immune deficiency are now exposed to simian retroviral infection. Possible superinfection with an SIV, STLV, or simian foamy virus could increase viral adaptation, replication, or even recombination between SIV and HIV, with unknown pathogenicity [1, 6]. Microbiological surveillance of high-risk populations such as hunters will be crucial to prevent or better contain such possible events.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. The authors thank Philippe Engandja for technical help. The International Centre for Medical Research in Franceville is funded by the Gabonese Government, Total Gabon and the French Foreign Ministry.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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