New Strain of Human T Lymphotropic Virus (HTLV) Type 3 in a Pygmy from Cameroon with Peculiar HTLV Serologic Results

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A search for human T lymphotropic virus (HTLV) types 1 and 2 and related viruses was performed by serological and molecular means on samples obtained from 421 adult villagers from the southern Cameroon forest areas. One individual (a 56-year-old Baka Pygmy hunter) was found to be HTLV-3 infected; however, there was a low proviral load in blood cells. Complete sequence analysis of this virus (HTLV-$3_{Lobak18}$) indicated a close relationship to human HTLV-3_{Pyl43} and simian STLV-3_{CTO604} strains. Plasma samples from Lobak18, the HTLV-3 infected individual, exhibited a peculiar "HTLV-2–like" pattern on Western blot analysis and were serologically untypeable by line immunoassay. These results were different from those for the 2 previously reported HTLV-3 strains, raising questions about serological confirmation of infection with such retroviruses.

Human T cell lymphotropic virus (HTLV) type 1 and HTLV-2, as well as their simian counterparts (STLV-1 and STLV-2), belong to the primate T cell lymphotropic viruses (PTLVs), which share some common epidemiological and biological features. In 1994, a third STLV type, originally named STLV-L, was discovered in a baboon *(Papio hamadryas)* from Eritrea [1]. This virus ($STLV_{PH969}$), now considered the STLV-3 prototype, exhibited only 62% and 64% nucleotide-level similarity with HTLV-1 and HTLV-2, respectively [1, 2]. Since 2000, there have been 9 other STLV-3 strains identified in African nonhuman primates (NHP) [3–10]. *In natura,* STLV-3 infects a number of species, including *Papio, Cercopithecus, Cercocebus,* and *Theropithecus* [1–10]. These monkeys have a wide geographical distribution in Africa (east, central, and west) and live in very diverse ecosystems (desert, tropical rain forest, and savanna). In the context of the known interspecies transmission that has occurred between STLV-1–infected NHP and humans, leading to the present distribution of HTLV-1, it was tempting to speculate that some HTLV strains exist in humans that are related to STLV-3. The search for such strains led to the recent discovery of 2 related strains of a new human HTLV type (HTLV-3_{Pyl43} and HTLV-3_{2026ND}) that was named HTLV-3 [11, 12]. These 2 HTLV-3 strains were discovered in persons living in Cameroon, a country in central Africa where a highly diverse set of retroviruses exists in both humans and NHP [11, 12].

Interestingly, the HTLV serological results reported for the 2 HTLV-3–infected individuals were rather different, and the 2 strains exhibited roughly 12% nucleotide divergence [11–14]. Molecularly, although the sequence of Pyl43 is very closely related to some STLV-3 virus strains (especially the STLV-3_{CTO604} strain) found in monkeys currently living in the rain forest area of southern Cameroon, the other HTLV-3 (2026ND) is more distantly related to all the other currently known STLV-3 strains [3–10, 13, 14].

Methods. To get new insight into the origin, distribution, genetic diversity, and serological pattern of such HTLV-3 strains, we searched for other HTLV-3 strains in a new series of samples originating from Cameroonian inhabitants. The current study was performed on samples from 421 adults (age >18 years); 294 samples were from Baka Pygmies (mean age, 51 years; 130 women and 164 men), and 127 were from Bantus (mean age, 45 years; 55 women and 72 men) living in remote villages in the rain forest area of southern Cameroon (the Lomié

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region). This survey was approved by national authorities (the Ministry of Health and the Ethics Committee of Cameroon) and local authorities (the village chief); written informed consent was obtained from each individual included in the study.

All 421 plasma samples were tested with a confirmatory Western blot assay (HTLV-1 Blot 2.4; MP Biomedicals Singapore) without any prior initial screening. High-molecular-weight DNA was extracted from buffy-coat from the 421 peripheral blood samples and was subjected to polymerase chain reaction (PCR) that used human β-globin–specific primers, to ensure that DNA was amplifiable. The samples were then subjected to nested PCR amplifying a *tax* region (out, PTLVTPG-PGTAXR1; in, PH2Rrev-PTGAXR2) and 2 *pol* regions (out, PGPOLF1-PGPOLR1 and in, PGPOLF2-PGPOLR2; out, SCPOL1outse-SCPOL1outas and in, SCPOL1inse-SCPOL1outas) that are highly conserved in all PTLVs [11–13]. In addition, nested PCR that allowed the amplification of all known PTLV-3 strains (out, LTR62se-Gag856as; in, LTR111se-LTR716as) was performed, as described elsewhere [13].

Results. Twelve plasma samples exhibited a typical HTLV-1 Western blot pattern (strong reactivity to p19 and p24 gag proteins, with reactivity to p19 greater than that to p24, as well as reactivity to the recombinant protein GD21 and the gp46 HTLV-1–specific peptide MTA-1). None had a typical HTLV-2 Western blot pattern (i.e., reactivity to p24 greater than that to p19, as well as reactivity to GD21 and to the gp46 HTLV-2–specific peptide K55), and 152 plasma samples displayed an indeterminate Western blot pattern. The other 257 plasma samples were seronegative for HTLV by Western blot assay.

Sequence analysis of the Tax PCR products confirmed the presence of HTLV-1 in the DNA samples originating from the 12 persons with HTLV-1–positive Western blot results. Among the HTLV-seronegative and HTLV-indeterminate samples, all but 1 tested PCR negative for HTLV with the 4 nested PCRs. The DNA of this individual (Lobak18) was PCR positive for HTLV when tested with the *tax* and *pol* generic primers, as well as when tested with the PTLV-3–specific primers. Sequence analysis confirmed that this person was infected with a strain of HTLV-3.

Lobak18 is a 56-year-old Baka Pygmy with a peculiar HTLV Western blot serological pattern (figure 1). This pattern resembles HTLV-2, as the HTLV 2.4 Western blot profile displayed reactivity against p19 and p24 Gag proteins, as well as against GD21 (env gp21) and K55 (HTLV-2 env peptide). However, it is not a typical HTLV-2 pattern, because a typical HTLV-2–positive sample displays very strong p24 reactivity and the reactivity against p19 is weak or even absent (see figure 1). This is not the pattern of HTLV-$3_{Lobak18}$. Furthermore, the line immunoassay (LIA, INNO-LIA; Immunogenetics Belgium) showed a weak positive but untypeable HTLV profile, with faint reactivity against HTLV-1 and HTLV-2 gp21 and gp46 envelope peptides, and strong reactivity against HTLV-1 and 2 gag p19 but without HTLV-2–specific reactivity against gp46II and reactivity against p24. The same pattern was observed in a series of 2 samples collected 4 months apart (figure 1). Interestingly, the plasma from this individual also contained antibodies directed against the Tax3 protein, as demonstrated by Western blot analysis that used purified STLV-3 Tax3 as a source of antigen (data not shown). The wife of Lobak18 was HTLV seronegative by Western blot assay.

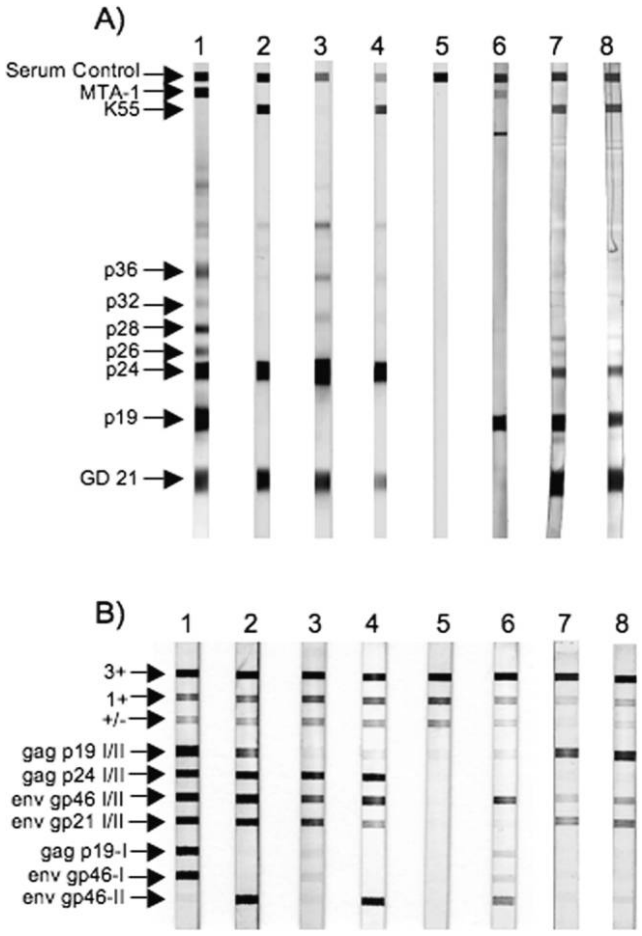

Figure 1. Serologic pattern of the individual infected by human T lymphotropic virus type 3 strain HTLV-$3_{Lobak18}$. *A,* Western blot analysis (HTLV-I Blot 2.4; MP Biomedicals Singapore); *B,* Line immunoassay (LIA, INNO-LIA; Immunogenetics Belgium). *Lane 1,* HTLV-1–positive control; *lane 2,* HTLV-2–positive control; *lanes 3* and *4,* simian T lymphotropic virus (STLV) strains (STLV-$3_{CTO-604}$ and STLV-3_{PPAF3}, respectively); *lane 5,* control negative for HTLV-1 and 2; *lane 6,* HTLV-3_{Pyl43} strain; *lanes 7* and *8,* HTLV-$3_{Lobak18}$ from 2 sequential plasma samples from Lobak18.

Lobak18 is a hunter, as are the great majority of the Baka Pygmy men. He frequently butchered wild game (including several monkey species), but did not recall any serious events that might have led to a wound or bite that involved blood, saliva, or other tissue fluids from a monkey.

A series of 14 successive nested PCR amplifications allowed the amplification and cloning of the complete HTLV-$3_{Lobak18}$ provirus (GenBank accession number, EU649782). The overall genetic organization of HTLV-$3_{Lobak18}$ is similar to that of other

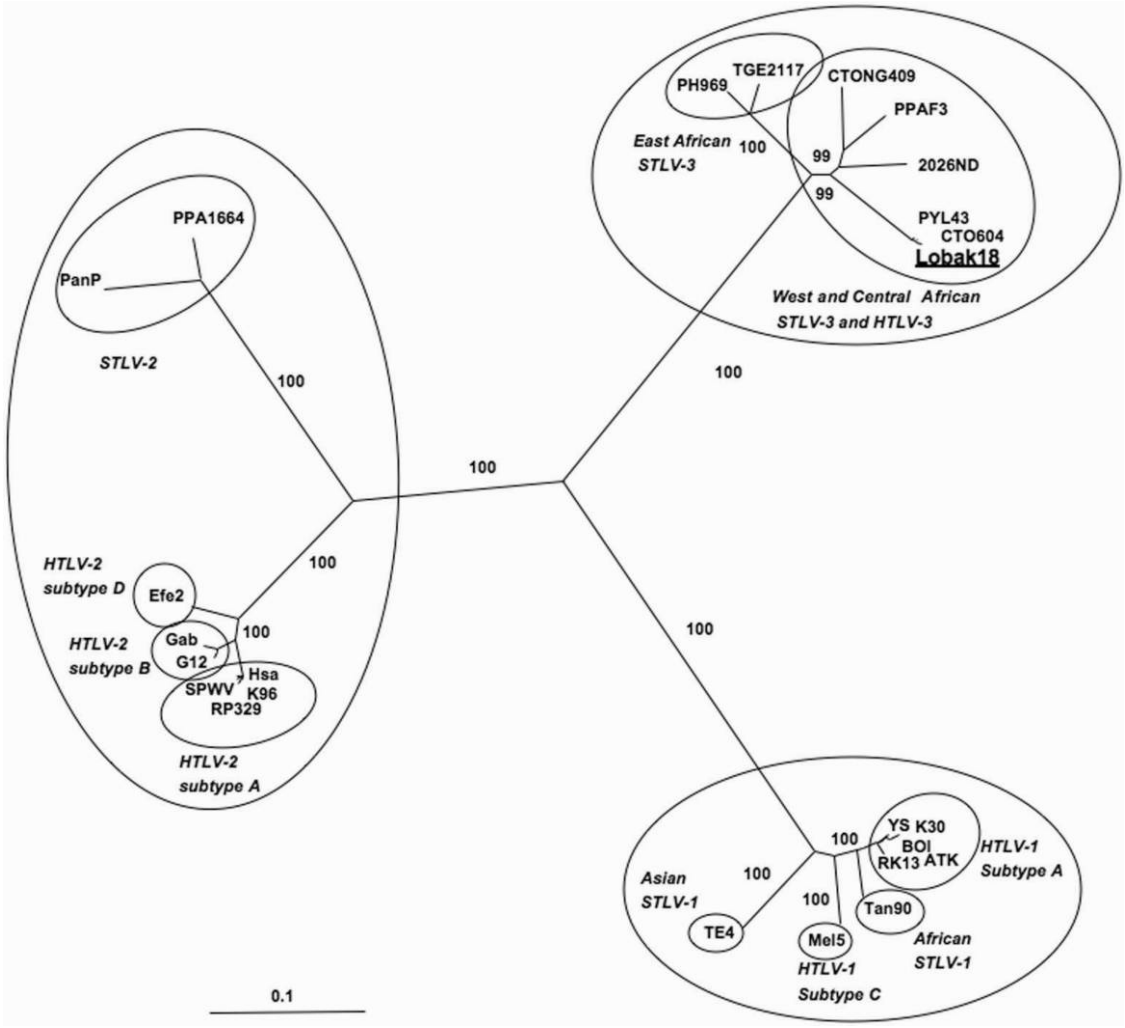

Figure 2. Unrooted phylogenetic tree generated with the neighbor-joining method (performed in PAUP [v4.0b10]), based on concatenated *gag-pol-env-tax* genes from the strain of human T lymphotropic virus (HTLV) type 3 recovered from Lobak18 (HTLV-$3_{Lobak18}$) and complete primate T cell lymphotropic viruses sequences available in GenBank. The bootstrap values (1000 replicates) are indicated on the branches of the tree. The branch length is drawn to scale, and the bar indicates 0.1 nucleotide replacements per site. STLV, simian T lymphotropic virus.

HTLV-3 and STLV-3 strains, including the presence of the *gag, pro, pol, env, tax,* and *rex* genes and only two 21-bp repeat sequences in the long terminal repeat. Interestingly, the 366-bp deletion previously reported for the HTLV-3_{Pyl43} strain is absent from HTLV-$3_{Lobak18}$ provirus [13]. Nucleotide sequence comparison revealed that HTLV-$3_{Lobak18}$ is closely related to STLV-3_{CTO604} (99.21% identity) and HTLV-3_{Pyl43} (99.27% identity if the 366-bp deletion is omitted for the calculation), whereas it exhibited an 11.64% divergence from the HTLV-3_{2026ND} strain.

Phylogenetic analyses based on the concatenated *gag-pol-env-tax* genes clearly demonstrated that HTLV-$3_{Lobak18}$ belonged to the PTLV-3 west and central African group (figure 2). It is worthwhile to note that among the 4 PTLV-3 strains from Cameroon for which the complete proviral sequence is available, the 3 most closely related strains (STLV-3_{CTO604}, HTLV-3_{Pyl43}, and HTLV-$3_{Lobak18}$) originated from the southern Cameroon area, whereas the more divergent strain (HTLV-3_{2026ND}) originated from an area located approximately 500 km north.

Using a semiquantitative PCR assay, we quantified the proviral load of HTLV-$3_{Lobak18}$. The virus was present at 3 copies in 150,000 peripheral blood mononuclear cells (data not shown). We also tried to isolate the HTLV-$3_{Lobak18}$ virus. To this end, $CD4^+$ cells were isolated and cultured in the presence of interleukin-2 after an initial stimulation with phytohemagglutinin or protein A. The $CD4^-$ fraction was also cultured in the same conditions. After 10 days of culture, HTLV-3 proviral DNA was still detectable in both the $CD4^+$ and the $CD4^-$ populations (3 copies in 150,000 cells). The results were similar at day 43, although 30 copies were detected in the $CD4^-$ population. However, a stable cell line could not be obtained, and the different cell cultures eventually died after 4–5 months.

Discussion. We report here the detection and molecular characterization of a new HTLV-3 strain present in peripheral blood cells obtained from a Cameroonian Baka Pygmy whose plasma exhibited peculiar HTLV serologic results. Interestingly, the new HTLV-$3_{Lobak18}$ strain displays Western blot and line immunoassay profiles that are different from those of the 2 other HTLV-3 strains (HTLV-$3_{Pyl43}$and HTLV-3_{2026ND}) [12, 15]. In addition, the HTLV-$3_{Lobak18}$ serologic results are different from the serological patterns previously reported for most STLV-3 strains [3–9]. These monkey sera exhibit mainly an "HTLV-2–like" profile with clear reactivity against gag p24, as well as against GD21 (env gp21), whether or not associated with HTLV-2 env gp46 K55 (figure 1), whereas serologic results for HTLV-$3_{Lobak18}$ also display strong reactivity against gag p19 (figure 1). More surprisingly, the HTLV-$3_{Lobak18}$ serological pattern is also different from that of HTLV-3_{pyl43} and HTLV-3_{2026ND}. Indeed, HTLV-3_{pyl43} presented clear reactivity to gag p19 associated with a faint HTLV-1 env gp46 MTA-1 peptide, while serum samples from individuals infected with HTLV-3_{2026ND} contained antibodies against both gag p19 and p24, as well as against GD21 (env gp21) and the HTLV-1 env gp46 MTA-1 peptide (reactivity was faint for the latter). Taken together, these results clearly indicate that humans or monkeys infected by very closely related (or nearly identical) HTLV-3 and STLV-3 viruses can exhibit a large variety of Western blot and line immunoassay patterns. The reason for such findings remains unknown, and they might indicate, in some cases, a unique individual host response effect. These findings raise also important questions regarding the specific serological diagnosis of such infection in blood banks, and they indicate the need for improved additional serological tests that take into consideration all of the HTLV types and are able to differentiate among them [15].

Studies involving large populations are ongoing, especially among different ethnic groups living in Cameroon, the Central African Republic, and Gabon, with the goal of identifying additional HTLV-3 strains. Based on the fact that the 3 known HTLV-3 strains were discovered in 3 geographically distant areas and recovered from 3 different ethnic groups, and given the high prevalence of STLV-3 infection and wide diversity of STLV-3 strains in African monkeys, it is tempting to speculate that such viruses might be widespread in several areas of the African continent.

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