Simian T cell leukaemia virus type I subtype B in a wild-caught gorilla (*Gorilla gorilla gorilla*) and chimpanzee (*Pan troglodytes vellerosus*) from Cameroon

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A serological survey for human T cell leukaemia virus (HTLV)/simian T cell leukaemia virus (STLV) antibodies was performed in 61 wild-caught African apes, including five gorillas and 56 chimpanzees originating from south Cameroon. Two young animals, a gorilla (*Gorilla gorilla gorilla*) and a chimpanzee (*Pan troglodytes vellerosus*), exhibited a pattern of complete HTLV-I seroreactivity. Sequence comparison and phylogenetic analyses using the complete LTR (750 bp) and a 522 bp fragment of the env gene indicated the existence of two novel STLV-I strains, both of which belonged to HTLV-I/STLV-I molecular clade subtype B, specific to central Africa. These first STLV-I strains to be characterized in gorilla and chimpanzee were closely related to each other as well as to several HTLV-I strains originating from inhabitants of south Cameroon, including pygmies. Such findings reinforce the hypothesis of interspecies transmission of STLV-I to humans, leading to the present day distribution of HTLV-I in central African inhabitants.

Primate T lymphotropic virus type I (PTLV-I) (Guo et al., 1984; Watanabe et al., 1986, 1985) viruses include human T cell leukaemia virus type I (HTLV-I) and simian T cell leukaemia virus type I (STLV-I) (Miyoshi et al., 1982). STLV-I, which is endemic in many Old World monkey species (Ibrahim et al., 1995; Ishikawa et al., 1987), can cause an adult T cell leukaemia-like pathology in some infected monkeys. HTLV-I possesses a remarkable genetic stability and the few nucleotide substitutions observed among virus strains are specific to the geographic origin of the patient rather than to pathology (Gessain et al., 1992; Mahieux et al., 1997). Indeed, four major geographic subtypes (genotypes) have been reported: Cosmopolitan HTLV-I subtype A, central African HTLV-I subtype B, Melanesian HTLV-I subtype C and the more recently discovered subtype D present in central Africa.

The origin of most geographic HTLV-I subtypes appears to be linked to episodes of interspecies transmission between STLV-I-infected monkeys and humans, followed by variable periods of evolution in the human host (Mahieux et al., 1997; Salemi et al., 2000; Slattery et al., 1999; Van Dooren et al., 2001; Vandamme et al., 1998; Watanabe et al., 1985). Evidence of interspecies transmission episodes were described between HTLV-I subtype B and D strains from central African inhabitants and few STLV-I strains from wild-caught chimpanzees and mandrills, respectively (Koralnik et al., 1994; Mahieux et al., 1998; Nerrienet et al., 2001; Voevodin et al., 1997). Furthermore, two other STLV-I strains originating from a wild-caught mandrill from Gabon and a wild-caught *Cercocebus agilis* from Cameroon were found recently to be closely related to the Lib2 HTLV-I strain subtype F from an inhabitant from Gabon. This supports the notion of interspecies transmission (Mahieux et al., 1998; Nerrienet et al., 2001).

STLV-I is endemic in many African monkey (sub)species (Ishikawa et al., 1987; Saksena et al., 1994). However, among African apes, very few STLV-I strains have been characterized and all but two from captive animals in Japan, USA or The Netherlands. Among the chimpanzee species (*Pan troglodytes*), four main subspecies have been described: *P. t. verus* (west Africa), *P. t. troglodytes* and *P. t. vellerosus* (central Africa) and *P. t. schweinfurthii* (east Africa). Regarding STLV, the first STLV-I sequence from a chimpanzee (CH strain) was obtained from a captive animal in Japan (Watanabe et al., 1986). Later on, Koralnik et al. (1994) published three gp21 env sequences from captive chimpanzees (one originated from Sierra Leone but the precise origin of the other two was unknown), while Voevodin et al. (1997) reported the presence of STLV-I strains in chimpanzees held at the National Cancer Institute/
National Institutes of Health facilities in the USA. More recently, Niphuis et al. (2003) reported the presence of STLV-I strains in a large closed breeding colony of chimpanzees originating from west Africa, belonging to the P. t. verus subspecies, held in a primate centre in The Netherlands. Most of these env sequences, as well as one published recently by our group, from a wild-caught chimpanzee of the P. t. troglodytes subspecies (PTR-CAR875) in Cameroon, were slightly different to each other but belonged to the large HTLV-I/STLV-I molecular clade subtype B, specific to central Africa (Nerrienet et al., 2001). Furthermore, despite the rare reports of STLV-I seropositive gorillas (Blakeslee et al., 2001), P. t. verus chimpanzees originating from west Africa, belonging to STLV-I strains in a large closed breeding colony of chimpanzees originating from Cameroon. These animals (from two zoos and one sanctuary) originated from different areas of Cameroon (mostly the southern regions) and were, for the most part, kept initially as pets for a variable period of time (after their mothers had been killed by hunters). These animals were either brought to the zoos or sanctuary or were confiscated by the Ministry of Environment and Forestry (MINEF) and then taken to the sanctuary.

For all plasma samples tested, two serological screening tests [immunofluorescence analysis (IFA) on MT2 and C19 cells and ELISA (Platelia HTLV-1/2, Sanofi Diagnostic Pasteur)] revealed two positive and one borderline (or uncertain) specimens. These three samples were tested further by a confirmatory Western blot assay (HTLV-2-4, Diagnostic Biotechnology Singapore). Among them, two samples (PTR-CAM43 and GGO-CAM12) exhibited a complete HTLV-I pattern with antibody reactivities against both the p19 and p24 antigens, the recombinant protein GD21 and the HTLV-I-specific gp46 peptide MTA-1. The third one was negative by Western blot. These two samples were clearly positive by IFA, with titres on MT2 cells of 1/320 and 1/160, respectively. The two HTLV-I/STLV-I seropositive animals were PTR-CAM43, a 1-year-old female P. t. vellerosus (subspecies confirmed by DNA mitochondrial sequence analysis; data not shown), and GGO-CAM12, a 16-month-old female G. g. gorilla. Both primates originated from the southwestern and -eastern regions of Cameroon, respectively. Also, they were both STLV-I seropositive since their first HTLV serological test, performed 2 months and 1 month, respectively, after their arrival in the sanctuary/zoo.

PCR was performed on high molecular mass DNA extracted from the PBMCs of these two animals and from several HTLV-I-positive and -negative molecular controls. The complete LTR (755 bp) and a 522 bp fragment of the env gene (gp21) were amplified and sequenced, as described previously (Meertens et al., 2001). The two new LTR and env sequences determined herein were deposited in the National Center for Biotechnology Information database (Genbank accession nos AY263381–AY263384).

The genetic comparison of these two new sequences with the other published STLV-I and HTLV-I env sequences revealed some interesting findings: the novel chimpanzee strain (PTR-CAM43), the first one from a P. t. vellerosus subspecies, was closely related to the sequences of the HTLV-I/STLV-I molecular clade subtype B. Furthermore, it was more related to several HTLV-I than to most of the other chimpanzee viruses of this group. Thus, the novel PTR-CAM43 strain exhibited more than 98.5% similarity (and up to 99.4%) to several HTLV-I strains, including H2-4, B5-1 and G2-4 isolates, originating from inhabitants of south Cameroon, including one Baka Pygmy (Mahieux et al., 1997), while it shares only between 97 and 98% similarity with most of the other subtype B chimpanzee viruses. All of the latter viruses derived from captive animals, for most of which the precise origin was unknown, including recently reported sequences from a colony of P. t. verus captive in The Netherlands. However, it is worthwhile to note that the novel chimpanzee sequence PTR-CAM43 was slightly more related (98.4% similarity) to the only other available sequence (PTR-CAR875) from a wild-caught P. t. troglodytes also originating from Cameroon. Comparative analyses of LTR sequences revealed again some close similarities between the new chimpanzee STLV-I and some of the HTLV-I isolates, especially strain 12503 (98.2% similarity) originating from a pygmy living in the Central African Republic near the border of south Cameroon (Mahieux et al., 1997).

The env and LTR sequences from G. g. gorilla (GGO-CAM12), the first ones characterized in this species, were closely related to the novel PTR-CAM43 strain (98.6 and 97.8% similarity, respectively) and thus to some HTLV-I subtype B isolates, especially G2-4 (99% similarity in the env gene).

Phylogenetic analyses using the neighbour-joining method were performed on all available env and LTR STLV-I sequences from Africa as well as from several representative HTLV-I strains of the different subtypes described previously (Meertens et al., 2001; Salemi et al., 1998). Similar tree topologies were obtained for both genomic regions. Analyses of the trees (Figs 1 and 2) reinforced the close relationship of the two new STLV-I strains with some HTLV-I strains. Indeed, the two novel STLV-I isolates belong to the large subtype B clade, which comprises several HTLV-I isolates, mostly from central Africa, and very few STLV-I isolates. This was clear for the two genomic regions analysed (LTR and env) (Figs 1 and 2).

Regarding the STLV-I chimpanzee isolates, if we analyse all of the gp21 env and the few complete LTR available data (Koralnik et al., 1994; Nerrienet et al., 2001; Niphuis et al., 2003; Voedovin et al., 1997; Watanabe et al., 1986), we can make the following conclusions: three main subgroups of
Fig. 1. Rooted phylogenetic tree generated by the neighbour-joining method with a 522 bp fragment of the env gene encompassing most of the gp21 and the C terminus of gp46. The HTLV-I subtype C MEL5 prototype sequence was used as the outgroup. Bootstrap analysis was applied with 1000 data sets. Distance matrices were generated using the DNADIST program with Kimura's two-parameter method and 5-69 as the transition/transversion ratio. The values on the branches indicate frequencies of occurrence for 1000 trees. The two new STLV-I sequences (PTR-CAM43 and GGO-CAM12) were analysed with 114 STLV-I/HTLV-I sequences (available from GenBank). Branch lengths are proportional to the evolutionary distances between the taxa.
chimpanzee STLV-I strains appear to exist. The largest one of these three subgroups is represented by subtype B strains; the second comprises only few sequences related to some STLV-I isolates present in *C. aethiops* from central and western Africa; the last one, reported recently by Leendertz et al. (2003), is represented by a sole isolate from the Ivory Coast (PTR-Leo strain).

Among the first group, the different chimpanzee strains exhibit a slight level of genetic variation (range of 1.5–2.9% of nucleotide divergence in the gp21 *env* gene). It is clear that the chimpanzees from western Africa (*P. t. verus* subspecies) are infected by a closely related virus (clade with a high bootstrap value in the *env* tree). This virus is, however, slightly different from that found in the two wild-caught chimpanzees from central Africa, of the *P. t. troglodytes* and *P. t. vellerosus* subspecies.

In conclusion, our study demonstrates for the first time the presence of STLV-I subtype B in a wild-caught gorilla as well as in a wild-caught chimpanzee of the *P. t. vellerosus* subspecies. Because most of the 61 apes studied were taken from their natural ecosystem as very young animals, these infections most probably reflect mother-to-offspring transmission, thus suggesting that the level of STLV-I infection in a representative population of apes (including adults) in the wild might be higher than thought previously. However, only non-invasive serological and molecular studies performed on faeces and urine samples will permit new insights into the real prevalence, geographic and subspecies distribution of such ape viruses in the wild. Similar studies have been performed recently with success for simian immunodeficiency virus infection in feral chimpanzees (Santiago et al., 2002). These ongoing field studies will permit a better view of the real biodiversity of STLV-I infection, which is still far from being known. Finally, the findings in these two wild-caught apes of STLV-I, closely affiliated to HTLV-I present in inhabitants of the same geographical areas (south Cameroon), strongly reinforce the notion of interspecies spread.
transmission of STLV-I from primates to humans, leading to the present day distribution of HTLV-I in some inhabitants of these areas.

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