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Molecular Epidemiology of 58 New African Human T-Cell Leukemia Virus Type 1 (HTLV-1) Strains: Identification of a New and Distinct HTLV-1 Molecular Subtype in Central Africa and in Pygmies

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To gain new insights on the origin, evolution, and modes of dissemination of human T-cell leukemia virus type 1 (HTLV-1), we performed a molecular analysis of 58 new African HTLV-1 strains (18 from West Africa, 36 from Central Africa, and 4 from South Africa) originating from 13 countries. Of particular interest were eight strains from Pygmies of remote areas of Cameroon and the Central African Republic (CAR), considered to be the oldest inhabitants of these regions. Eight long-term activated T-cell lines producing HTLV-1 gag and env antigens were established from peripheral blood mononuclear cell cultures of HTLV-1 seropositive individuals, including three from Pygmies. A fragment of the *env* gene encompassing most of the gp21 transmembrane region was sequenced for the 58 new strains, while the complete long terminal repeat (LTR) region was sequenced for 9 strains, including 4 from Pygmies. Comparative sequence analyses and phylogenetic studies performed on both the *env* and LTR regions by the neighbor-joining and DNA parsimony methods demonstrated that all 22 strains from West and South Africa belong to the widespread cosmopolitan subtype (also called HTLV-1 subtype A). Within or alongside the previously described Zairian cluster (HTLV-1 subtype B), we discovered a number of new HTLV-1 variants forming different subgroups corresponding mainly to the geographical origins of the infected persons, Cameroon, Gabon, and Zaire. Six of the eight Pygmy strains clustered together within this Central African subtype, suggesting a common origin. Furthermore, three new strains (two originating from Pygmies from Cameroon and the CAR, respectively, and one from a Gabonese individual) were particularly divergent and formed a distinct new phylogenetic cluster, characterized by specific mutations and occupying in most analyses a unique phylogenetic position between the large Central African genotype (HTLV-1 subtype B) and the Melanesian subtype (HTLV-1 subtype C). We have tentatively named this new HTLV-1 genotype HTLV-1 subtype D. While the HTLV-1 subtype D strains were not closely related to any known African strain of simian T-cell leukemia virus type 1 (STLV-1), other Pygmy strains and some of the new Cameroonian and Gabonese HTLV-1 strains were very similar (>98% nucleotide identity) to chimpanzee STLV-1 strains, reinforcing the hypothesis of interspecies transmission between humans and monkeys in Central Africa.

Human T-cell leukemia virus type 1 (HTLV-1), the first human oncoretrovirus isolated in 1980 (62), is the etiologic agent of a malignant CD4 lymphoproliferation (adult T-cell leukemia [ATL]) (85) and of a chronic progressive neuromyelopathy (tropical spastic paraparesis/HTLV-1-associated myelopathy [TSP/HAM]) (22, 36). Furthermore, this virus has been associated with cases of uveitis, arthritis, polymyositis, and infective dermatitis in areas where HTLV-1 is endemic (5, 57). Among the 15 to 25 million HTLV-1-infected individuals living throughout the world, roughly 1 to 5% will develop ATL or TSP/HAM, depending on as-yet-unknown cofactors which could vary according to geographical locations (5, 57).

The HTLV-1 genome varies little among strains (18, 25, 27, 45, 63), and recent data indicate that viral replication via clonal expansion of infected cells rather than by reverse transcription could explain this remarkable genetic stability (81). In parallel, the molecular epidemiology of HTLV-1 proviruses has shown that the few nucleotide changes observed among strains were specific for the geographical origins of the patients. However, there are no consistent differences between strains from patients with ATL versus those from patients with TSP/HAM (7, 11, 25, 27, 28, 31, 37, 39, 45, 46, 51, 55, 60, 63, 68, 72, 75–78, 83). Based upon sequence and/or restriction fragment length polymorphism data from the *pol* and *env* genes and/or the long terminal repeat (LTR) of more than 250 different strains, there are three major geographical subtypes (or genotypes) (25, 27, 28, 48, 55, 67, 76, 78, 83) strongly supported by high bootstrap

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values in phylogenetic analysis. Each of these genotypes (cosmopolitan, HTLV-1 subtype A; Central African, HTLV-1 subtype B; and Melanesian, HTLV-1 subtype C) appears to have arisen from interspecies transmission between simian T-cell leukemia virus type 1 (STLV-1)-infected monkeys and humans followed by variable periods of evolution in the human host (28, 42, 47, 48, 65, 78).

Furthermore, careful sequence analyses have indicated that within or alongside these three main genotypes, there exist molecular subgroups defined by several specific mutations but not always supported by phylogenetic analyses (28, 55, 76, 77). While there is 2 to 8% variation between geographical subtypes, HTLV-1 quasispecies present within an individual vary by less than 0.5% (28).

Africa is considered the largest area where HTLV-1 is endemic, with an estimated 3 to 8 million infected persons (4, 5, 7, 13–16, 41, 43, 49, 53, 54, 57). However, HTLV-1 is not uniformly distributed in Africa, and clusters of HTLV-1 exist near areas of very low prevalence (7, 13, 41). The reasons for this uneven distribution remain unknown but may be linked to as-yet-unknown environmental, cultural, and/or genetic cofactors. Despite the high prevalence of HTLV-1 in Africa, data for the genetic structure of HTLV-1 strains from this continent are drawn predominantly from Zairian strains (7, 16, 23, 25, 49, 60), as well as strains from West Africa (25, 46) and North Africa (21). The Zairian strains represent the prototype Central African HTLV-1 genotype (HTLV-1 subtype B) (23, 25, 49, 63), while the other few known strains belong to the widely dispersed cosmopolitan genotype (HTLV-1 subtype A).

Studies of remote human populations have been instrumental in gaining new insights into the genetic diversity, origin, and evolution of both HTLV-1 and HTLV-2. The most divergent HTLV-1 genotype was isolated from aborigines living in Papua New Guinea and Australia (2, 24, 31, 70, 83, 84). Furthermore, the analysis of HTLV-2 isolates from Pygmy populations in Cameroon and Zaire have greatly modified our views on the origin of HTLV-2, which was until recently considered a New World virus restricted to Amerindian populations (19, 29, 32–35). Thus, the main goals of present study were (i) to isolate and characterize the genetic structure of HTLV-1 strains present in different Pygmy groups living in remote areas of Cameroon and the Central African Republic (CAR) and considered to be the oldest inhabitants of equatorial Africa, (ii) to perform a phylogenetic analysis comparing these Pygmy strains to all the other African strains available and especially to the 50 new non-Pygmy strains originating from 13 countries which were also characterized in the present study, and (iii) to compare all 58 new African HTLV-1 strains to available African STLV-1 strains to test the hypothesis of interspecies transmission.

MATERIALS AND METHODS

Serological tests. For serum samples obtained through the Pasteur Institute International Network (26, 40, 53, 54), the following two tests were used to detect HTLV-1 antibodies: an enzyme-linked immunosorbent assay (ELISA; Diagnostic Biotechnology, Singapore, Singapore) and an indirect immunofluorescence assay (IFA) using MT2 HTLV-1-producing cells and C19 HTLV-2-producing cells. IFA was also used to determine the titers of HTLV-1 antibodies. For confirmation, we used a Western blot (immunoblot) assay (HTLV2-3; Diagnostic Biotechnology) which contained disrupted HTLV-1 virions, a recombinant gp21 (rgp21) protein, and MT4-1, an HTLV-1-specific peptide corresponding to residues 169 through 209 of the gp46 glycoprotein (8, 38). Stringent Western blot confirmatory criteria were used, and a serum sample was considered to be HTLV-1 positive only if it exhibited antibodies against both rgp21 and MTA-1 as well as against both p19 and p24.

Viral isolation and immunological studies. After informed consent was obtained, a venipuncture was performed for HTLV-1-seropositive individuals and peripheral blood mononuclear cells (PBMCs) were separated and sent frozen on dry ice to our laboratory (Unité d'Epidémiologie des Virus Oncogènes) at the Pasteur Institute, Paris, France. In some cases, PBMCs were put in culture and maintained in a humidified 5% CO_2 air atmosphere with biweekly changes of RPMI 1640 medium (Whittaker Bioproducts, Verviers, Belgium) supplemented with 20% heat-inactivated fetal calf serum, 10% interleukin 2 (Boehringer, Mannheim, Germany), 1% L-Gln, and 1% penicillin-streptomycin (GIBCO BRL, Paisley, Scotland). During the first 3 days, cells were stimulated with phytohemagglutinin (DIFCO) at 2 μg/10^6 cells. An IFA (29) was performed with cultured cells to detect viral antigen expression by using either mouse monoclonal antibodies directed against HTLV-1 p19 or p24 (Cambridge Biotech) or polyclonal sera from HTLV-1-infected individuals.

Flow cytometric analysis (kindly performed by F. Valensi, Necker Hospital, Paris, France) of the cultured cell lines was performed with a series of monoclonal antibodies directed against human T-cell and B-cell antigens as previously described (30).

The production of the p24 core antigen in the culture supernatant was measured by an antigen capture ELISA that detects HTLV-1 and -2 and STLV-1 p24 (HTLV antigen assay; Coulter). The production of the p19 core antigen in the culture supernatant was measured by an antigen capture ELISA that detects HTLV-1 and -2 and STLV-1 p19 (Retrotek HTLVp19; Cellular Products).

PCR. PCR was carried out as previously described (25, 29, 42). Briefly, high-molecular-weight DNA was extracted from PBMCs before or during cell culture by a classical phenol-chloroform technique. Each reaction mixture contained 1.5 μg of DNA, 0.2 mM deoxynucleoside triphosphate mix (Boehringer), 10 μl of a 10× reaction buffer, 25 mM (each) oligonucleotide primers, and 2.5 U of *Taq* DNA Polymerase (Perkin-Elmer Cetus) in a total volume of 100 μl. For each sample, we used HTLV-1-specific primers and appropriate probes. All the upstream (or sense) primers were synthesized with the linker sequence, TTTGAG CGGCCGC, containing the restriction site for *Not*I, and all the downstream (or antisense) primers were synthesized with the linker sequence, ACTTAGAAT TC, carrying the restriction site for *Eco*RI.

***env* amplification.** A seminested PCR was done by using Env1 and Env22 as outer primers and Env1 and Env2 as inner primers. Two microliters of the initial PCR mixture was used for the second PCR run, and the reaction was analyzed on a 1.5% agarose (ultrapure agarose; Bethesda Research Laboratories) gel. The sequences of these *env*-specific primers are as follows: Env1, 5′ (6044) TCAAG CTATAGTCTCCTCCCCCTG (6064) 3′ (sense); Env2, 5′ (6613) GGGAGGT GTCGTAGCTGCAGGAGG (6590) 3′ (antisense); and Env22, 5′ (6795) GGC GAGGTGGAGTCCTTGGAGGC (6773) 3′ (antisense). The probes were PR env, 5′ CCTTGAGAATCGAGTCCTGAC 3′, and PE1-2, 5′ CAGACGAGGC CTTGATCTCCTGTT 3′.

LTR amplification. Two different approaches were carried out for cloning the entire LTR. (i) In the case of long-term cultures with a high HTLV-1 load, a single PCR was performed with first a 512-bp (positions 8221 to 8733) amplified fragment (P23ACDF/LTRU5E) encompassing the 3′ end of the pX region and the first 461 bases of the LTR U3 region and part of the R region and a second 734-bp (positions 44 to 778) overlapping fragment (P3LTR/5PLTR) encompassing part of the R region and all of the U5 region.

(ii) For the analysis of uncultured PBMCs with a low HTLV-1 load, a seminested PCR was performed. The first fragment (8255/LTRU5E) of 467 bp (positions 8266 to 8733) was amplified, and 2 μl of this initial PCR mixture was used for the second run, generating a fragment (8255/420LTR) of 433 bp (positions 8266 to 8699). The second fragment (Enh280/5PLTR) of 477 bp (positions 301 to 778) was amplified, and 2 μl of the initial PCR mixture was used for the second PCR run, amplifying an inner fragment (TATAbox/5PLTR) of 418 bp (positions 360 to 778).

The sequences of these LTR-specific primers are as follows: P23ACDF, CAT TTCTACTCTCACACGGCCTCATACAGTACTCTT (sense); P3LTR, TGAC AATGACCATGAGCCCCA (sense); LTRU5E, CGCAGTTCAGGAGGCAC CAC (antisense); 5PLTR, TCCCGGACGAGCCCCCAA (antisense); Enh280, TGACGACAACCCCTCACCTCAA (sense); TATAbox, CAGGAGTCTATA AAAGCGTGG (sense); 8255, TTGAAGAATACACCAACATCCC (sense); and 420LTR, GAACGCGACTCAACCGGCCGTGGAT (antisense). The probes were RPX2, CCATCCACGCCGGTTGAGTCGCGT, and 361, TCCTT CACGCGCCCGCCGC .

For all PCR experiments, the amplification mixes were made in a special room physically separated from the laboratory. After denaturation at 94°C for 5 min, the reaction mixtures containing DNA were cycled 35 times (*env*) or 40 times (LTR) at 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min. An extension of 2 s per cycle was realized with an extension of 10 min on the last cycle.

Nucleotide sequencing. Purified PCR products were obtained after phenol-chloroform extraction and ethanol precipitation and then digested with 10 U of *Eco*RI and *Not*I (Boehringer), respectively. The digested DNA was purified by centrifugal filtration (Millipore) inserted into a linear *Eco*RI-*Not*I pBlueScript vector, and molecularly cloned. Positive clones were selected by using ^{32}P-labelled oligoprobes derived from the HTLV-1 ATK sequence. For each sample, the plasmid DNA from one or two positive recombinant clones was extracted, purified (Midiprep; Qiagen), and sequenced (Sequenase, version 2.0; U.S. Biochemicals).

Phylogenetic analyses. Several steps were carried out in order to derive phylogenetic trees from the original set of sequences. The alignment of multiple sequences, including the new HTLV-1 *env* and LTR sequences and most of the HTLV-1 and STLV-1 sequences previously published, was performed by using

the CLUSTAL W program (74). The resulting aligned sequences were submitted to different programs of the PHYLIP package (version 3.52c; Joseph Felsenstein, University of Washington [e-mail: joe@genetics.washington.edu]) and of the MEGA program (version 1.01 5; S. Kumar, K. Tamura, and N. Nei, Pennsylvania State University, University Park, Pa.).

The following two methods were used to generate phylogenetic trees: the maximum parsimony method using the DNAPARS program and the neighbor-joining (NJ; NEIGHBOR program method) (64) using the modified NJ approach (59). This technique uses the modified Kimura two-parameter distance, which is a maximum-likehood estimate of Kimura distance for a fixed value of alpha/beta (transition/transversion ratio = 2). The SEQBOOT program was carried out to generate 100 to 500 data sets that are random resampled versions of the previously aligned sequences. For both maximum parsimony and NJ methods, a consensus tree was constructed by using the CONSENSE program with the majority-rule criteria. The distance-based tree was constructed with the MEGA program by the NJ method with the Kimura two-parameter model and 500 replicates (bootstrap) in order to test the reliability of the final tree topology.

The diversity values within and between subtypes were obtained by comparing pairs of nucleotide sequences and calculating for each one a pairwise distance, defined as the number of mismatches between the two sequences divided by the length of their alignment. The within-subtype diversity was based on the comparison of sequences from the same subtype. The diversity between two subtypes was generated by comparing all the sequences from one subtype with all the sequences from the other subtype. The mutation tree was designed to show the nucleotide mutations specific to subtypes. Nine subgroups were defined within the four subtypes; for each of these subgroups, the proportion of the most frequent nucleotides per position was calculated. Only the nucleotides common to more than 80% of the sequences of a subgroup were taken into account as subgroup-specific nucleotides. The positions where the subgroup-specific nucleotides differ were associated to the corresponding branch in the HTLV tree.

Nucleotide sequence accession numbers. The GenBank accession numbers for the 58 new gp21 *env* clones are L45695 through L45699, L46600 through L46632, L46636 through L46638, L46640 through L46650, L48558 through L48561, L76414, and L76415. The GenBank accession numbers for the nine new entire-LTR clones are L76305 through L76312 and L77212.

RESULTS

Subjects and specimens. Seroepidemiological studies were performed between 1991 and 1994 by the HTLV-1 network of the Pasteur Institute International Network. Seventy-two HTLV-1-seropositive samples were detected among roughly 10,000 serum and plasma samples of individuals living in Cameroon (52a, 53), the CAR (40), and Senegal (53a, 54). Most of these 72 samples exhibited a strong Western blot reactivity directed against specific HTLV-1 *gag*-encoded proteins (p19, p24, and pr53), *env*-encoded proteins rgp21 and gp46, and the MTA1 peptide specific for HTLV-1 gp46. After informed consent was obtained, a venipuncture of 10 to 20 ml of heparinized blood was performed on 33 of these HTLV-1-seropositive Africans in order to study the genetic structure of the HTLV-1 strains present in their PBMCs. Furthermore, through different French and international collaborations, we obtained frozen PBMCs or high-molecular-weight DNAs of another 25 HTLV-1-seropositive individuals. Thus, 58 new HTLV-1 samples originating from 13 African countries were available for this study (Table 1). They belonged either to 24 patients with HTLV-1-related diseases (17 with TSP/HAM and 7 with ATL) or to 34 HTLV-1-seropositive persons (either asymptomatic or with a disease not linked to HTLV-1 infection). As seen in Table 1, there were 33 females with a mean age of 45 years and 25 males with a mean age of 44.5 years. PH153 was the asymptomatic husband of patient PH121, who was diagnosed as having TSP/HAM. GAB3 and GAB4 were two brothers with TSP/HAM (14). Among these 58 samples, 8 originated from Pygmies living in remote areas of the CAR (Lobaye region) or Southern Cameroon (Table 2). Three of the Pygmies from the CAR (12503, 12504, and 12505) belonged to the Nzakara tribe family and were husband, wife, and son (26), respectively, while two Baka Pygmies from Cameroon were husband (H23) and wife (H24).

Viral isolation. PBMCs of 15 HTLV-1-seropositive persons (including 8 Pygmies) were cultured in the presence of interleukin 2. Eight long-term cultures were established (three of them from Pygmies), and the surface phenotype of these cell lines, as determined by flow cytometric analysis, demonstrated that these cells were of T-cell lineage with the expression of CD2, CD3, CD4, CD5, and activation markers (CD25 and HLA-DR) without B-cell markers (CD19 and CD20). In three cases, there was also expression of CD8 antigens at a significant level (data not shown). The level of expression of HTLV-1 antigens in cell cultures varied with the length of culture. No or minimal expression of HTLV-1 antigens was detected in short-term cultures (less than 1 month). After 45 days of culture, polyclonal sera from patients with TSP/HAM and murine monoclonal antibodies against p19 and p24 recognized 1 to 5% of cells. At 3 months of culture, 20 to 50% of cells expressed HTLV-1 antigens. The proportion of positive cells with polyclonal HTLV-1 sera was always higher than that with p19 and p24 monoclonal antibodies. The supernatants of cell lines were highly positive in antigen capture assays capable of detecting HTLV-1 p19 and/or p24 core antigens (data not shown).

Nucleic acid studies. (i) Sequence analysis of a fragment of gp21 *env*. HTLV-1 proviral sequences were detected by PCR in the DNA extracted from uncultured PBMCs in most of these 58 cases or from short- or long-term cultured cells of the few remaining HTLV-1-seropositive individuals by seminested PCR with Env1 and Env2 primers as the inner set. The sequences of these amplified fragments, corresponding to the carboxy terminus of gp46 and the majority of gp21, were determined for the 58 samples, representing a total of 30,276 bases analyzed. Within these 522-bp fragments, there were 106 mutation sites consisting of nucleotide substitutions; no deletions or insertions were found. Although the sequences of the 58 gp21 *env* fragments were highly related to each other, they exhibited up to 5.2% nucleotide divergence between themselves. The strains from the Senegalese couple (PH121 and PH153) were almost identical (99.6% similarity), suggesting possible sexual transmission of the same virus. The two brothers from Gabon with TSP/HAM also had a very similar HTLV-1 genotype (99.6%). While the three isolates from the CAR Pygmy family (12503, 12504, and 12505) were nearly identical (99.6 to 99.8%), the two isolates (H23 and H24) from the Cameroonian Pygmy couple were more divergent, exhibiting 2.5% variation (13 substitutions) between themselves. This unexpected discrepancy (found on uncultured ex vivo PBMC DNA) was confirmed by sequencing the gp21 *env* gene of the H24 established cell line. The 22 proviral sequences from West Africa and South Africa were very similar to each other, exhibiting 0 to 2% (maximum, 2.9%) sequence variation, with the exception of three strains (PH52, PH111, and PH122). One of these three, PH52 from the Ivory Coast, was the most divergent, exhibiting only 96 to 97% similarity with a few of the strains in this group. PH111 (also from the Ivory Coast) and PH122 (from Senegal) were also slightly divergent (96.7 to 97.8%) but were more closely related to PH52 (98.2 to 98.5%). In contrast, the genetic variability was higher among Central African strains, with a divergence ranging from 0 to 5.2%. Some strains were nearly identical: for example, four of the five new Zairian strains (I59, K361, K435, and K418) had more than 99.4% sequence similarity between themselves. Interestingly, six of the eight new Pygmy strains were very closely related (more than 99% similar) despite three different geographical origins. Four of them (010102, 12503, 12504, and 12505) lived in the Lobaye region of the CAR, and the last two (H24 and pyg294cult) lived in two areas of Cameroon 300 km apart. Only two other strains in the study (B51 and 839), also

TABLE 1. Epidemiological and clinical statuses of 58 HTLV-1-infected Africans for whom a 522-bp fragment of HTLV-1 gp21 was amplified and sequenced

Sample	Age (yr)/sex[a]	Origin	Clinical status[b]
pyg19	55/M	CAR	Healthy carrier
010102	35/F	CAR	Healthy carrier
12503	60/M	CAR	Healthy carrier
12504	50/F	CAR	Healthy carrier
12505	14/M	CAR	Healthy carrier
H23	44/M	Cameroon	Healthy carrier
H24	29/F	Cameroon	Healthy carrier
pyg294cult	70/M	Cameroon	Healthy carrier
PH120	28/F	Senegal	Prost. HIV-2 positive
PH121	47/F	Senegal	TSP/HAM
PH122	60/F	Senegal	TSP/HAM
PH123	/F	Senegal	Healthy carrier
PH153	51/M	Senegal	Healthy carrier
PH154	27/M	Senegal	Facial palsy HIV-1 positive
PH157	30/F	Senegal	Prost. HIV-2 positive
PH238	53/F	Senegal	Healthy carrier
PH239	61/M	Senegal	Healthy carrier
PH428maurit	23/M	Mauritania	ATL
PH333	38/M	Mauritania	*Histoplasmosis*
PH482guinbiss	53/M	Guinea-Bissau	ATL
PH71	45/M	Mali	ATL
PH52	/M	Ivory Coast	ATL
PH111	40/M	Ivory Coast	TSP/HAM HIV-1 positive
PH184	30/F	Burkina Faso	Healthy carrier
PH185	27/F	Burkina Faso	Healthy carrier
PH186	25/F	Burkina Faso	Healthy carrier
PH179	38/F	Chad	TSP/HAM
BCAM	64/F	Cameroon	Healthy carrier
716	62/F	Cameroon	Healthy carrier
839	43/F	Cameroon	Healthy carrier
PH240	29/F	Cameroon	HIV-1 positive
T49	52/F	Cameroon	Healthy carrier
B5-1	81/M	Cameroon	Healthy carrier
G2-4	31/F	Cameroon	Healthy carrier
BSABE	46/F	CAR	TSP/HAM
PH63	34/M	Congo	TSP/HAM
IGOU	/F	Gabon	ATL
GAB2	35/F	Gabon	ATL
GAB7	52/F	Gabon	ATL
GAB3	>60/M	Gabon	TSP/HAM
GAB4	>65/M	Gabon	TSP/HAM
2307	60/F	Gabon	Healthy carrier
2313	55/F	Gabon	Healthy carrier
2316	37/F	Gabon	Healthy carrier
2318	13/M	Gabon	Carrier
PH236	70/F	Gabon	TSP/HAM
27HO1	/F	Gabon	
SDen	/M	Gabon	TSP/HAM
K361	17/M	Zaire	NSP
K418	60/M	Zaire	Stroke
K435	16/M	Zaire	TSP/HAM
PH198	54/F	Zaire	TSP/HAM
MAS	56/F	Zaire	TSP/HAM
I.59	100/F	Zaire	Leprosy
afs911	45/F	South Africa	TSP/HAM
afs3	44/F	South Africa	TSP/HAM
afs2	40/M	South Africa	TSP/HAM
afs1	61/M	South Africa	TSP/HAM

[a] F, female; M, male.
[b] Prost., prostitute; HIV-1 and -2, human immunodeficiency virus types 1 and 2, respectively; NSP, nonspastic paraparesis.

from southern Cameroon, were closely related (>99.2% similarity) to these six Pygmy strains. On the other hand, four strains were different, exhibiting 2.2 to 5.2% of nucleotide difference to all of the other Central African strains. They included two strains from Pygmies (H23 and pyg19) living in Cameroon and the CAR, respectively, one from a non-Pygmy individual (2318) from East Gabon, and one from a patient with TSP/HAM (BSABE) living in the CAR.

In a second step, these *env* sequences were compared to all available HTLV-1 and STLV-1 sequences of African origin.

TABLE 2. Epidemiological statuses of eight HTLV-1-seropositive asymptomatic Pygmies from the CAR and Cameroon

Case	Age (yr)/sex[a]	Country	Region	Ethnic origin	Clinical status	IFA titer[b]		Sequence result[c]		
						MT2	C19	Western blot[d]	LTR	gp21[e]
pyg19	55/M	CAR	Lobaye	Biaka	Healthy carrier	1/20	1/20	+	+	+
010102	35/F	CAR	Lobaye	Nzakara	Healthy carrier	1/640	1/80	+	ND	+
12503	60/M	CAR	Lobaye	Nzakara	Healthy carrier	1/1,280	1/640	+	+	+
12504	50/F	CAR	Lobaye	Nzakara	Healthy carrier	1/1,280	1/160	+	ND	+
12505	14/M	CAR	Lobaye	Nzakara	Healthy carrier	1/1,280	1/40	+	ND	+
H23	44/M	Cameroon	South	Baka	Healthy carrier	1/40	1/20	+	+	+
H24	29/F	Cameroon	South	Baka	Healthy carrier	1/640	1/160	+	+	+
pyg294cult	70/M	Cameroon	South	Bakola	Healthy carrier	1/2,560	1/80	+	ND	+

[a] F, female; M, male.
[b] Reciprocal of last dilution showing positive immunofluorescence on acetone-fixed MT2-CEM and C19-CEM cells.
[c] +, positive result; ND, not done.
[d] Results obtained by HTLV2-3 assay (Diagnostic Biotechnology).
[e] Results obtained by seminested PCR using primers Env1 and Env22 followed by primers Env1 and Env2.

We found that the West and South African strains were closely related (97 to 99% similar) to the cosmopolitan (HTLV-1 subtype A) prototypes (ATK, MT2, and TSP1) (17, 37, 69), while most of the Central African strains were related (95.6 to 99.4%) to the previously published Zairian strains (HTLV-1 subtype B) (EL and Z15). Only 5 of the 46 Central African strains, including the only strain from Chad, were of the cosmopolitan subtype. In a comparison of STLV-1 sequences to cosmopolitan HTLV-1 subtype sequences, only PPAX528, a red baboon STLV-1 strain from West Africa, exhibited 96 to 97% nucleotide similarity to all the cosmopolitan strains. In contrast, there was striking similarities between some of the new Central African HTLV-1 and some chimpanzee STLV-1 strains (47). Such sequence similarity was very high (98.3 to 98.7%), especially between PTRX43 STLV-1 and some Pygmy isolates (010102, 12503, 12504, 12505, and pyg294cult) but also a few other strains from some individuals living either in the same forest area of southern Cameroon (B51) or in southern Gabon (GAB3 and GAB4).

After translation of these 58 nucleotide sequences (most of the mutations were silent because they occurred in the third codon position), the amino acids were aligned with the 17 homologous *env* sequences obtained by Koralnik et al. (47) from African HTLV-1 strains (Fig. 1). On the amino acid level, the following two subgroups emerged among these 75 African HTLV-1 strains (Fig. 1): one consisting mainly of all the West and South African strains, and the other one comprising the great majority (41 of 46) of the Central African strains, including all the Pygmy strains. While the first subgroup had an amino acid sequence identical to or very related to the cosmopolitan sequences, the second subgroup had at least one of the two specific Central African amino acid mutations, an M at position 330 and an R at position 344, as originally described by Koralnik et al. (47). Few other mutations corresponded to geographical origin. A Y at position 290 was present only in some Gabonese strains and one Cameroonian strain; a G at position 441 was present in some Zairian strains; and an I and an F were present at positions 368 and 454, respectively, in some of the cosmopolitan West African strains. None of the 58 new African sequences exhibited a threonine (instead of a glycine) at position 328, a mutation present only in Melanesian HTLV-1 strains as well as Asian STLV-1 and HTLV-2 strains.

(ii) Sequence analysis of the complete LTR. We also decided to study the LTR sequences of some of these strains for two reasons. (i) This fragment is known to be the most variable HTLV-1 region (45), especially in the U3 and U5 portions, thus allowing better phylogenetic discrimination among highly related strains than with gp21 sequence data (55). (ii) Despite the fact that only 38 complete HTLV-1 and STLV-1 LTR sequences were available compared to more than 150 for the gp21 *env* region, some African HTLV-1 and STLV-1 strains have been sequenced (often partially) only in this LTR region, thus allowing a more complete phylogenetic analysis of our new African strains. We chose to establish the complete LTR sequence of four Pygmy samples (H23, H24, pyg19, and 12503), four other strains from Central Africa (GAB7, PH236, SDen, and T49), and of one sample from South Africa (afs911) (Fig. 2). These LTR sequences were compared to all the available complete HTLV-1 and STLV-1 LTR sequences. It became obvious that the afs911 strain from South Africa belonged clearly to the cosmopolitan subtype, exhibiting only 0.9 to 3% nucleotide differences with all the strains in this group, while it differed by 4.3 to 5.5% from Central African strains. All Central African strains (except the two Pygmy strains pyg19 and H23) were closely related to each other, exhibiting 97.5 to 99.5% nucleotide similarity. Among those, strains PH236 and T49 were nearly identical, differing only 0.6% in sequence. The two Pygmy strains pyg19 and H23, although exhibiting 3.7% difference, were more closely related to each other than to any other HTLV-1 strain and differed from all the other Central African strains by 4.1 to 5.2%. Furthermore, this LTR analysis confirmed that the two samples, H23 and H24, isolated from sexual partners were two different strains, diverging by 4.8% in this genomic region. While no HTLV-1 strains clustered with STLV-1 AG (a simian isolate from a *Cercopithecus aethiops sabeus* from Kenya [80]), it was clear that STLV-1 CH (isolated from a chimpanzee from Sierra Leone [80]) belonged to the Central African group (more than 97% homology), which includes the previously published Zairian strains (EL, ITIS, MOMS, and MMMG) (48, 78) and five of the new ones (H24, PH236, SDen, T49, and GAB7). Neither the H23 nor pyg19 strain was closely related to STLV-1 AG or STLV-1 CH.

As seen in Fig. 2, the nine new LTR sequences aligned almost perfectly with the available HTLV-1 sequences, which include the HTLV-1 ATK prototype (69) and HTLV-1 Mel 5 (the most distant HTLV-1 of Melanesian origin [24]). Within Central African strains, the highest conservation of nucleotide sequence, compared to ATK, was observed in the R region (2.6 to 6.2% difference) while the variability was equivalent in the U3 (3.4 to 7.6%) and U5 (4.6 to 8%) regions. In the U3 region, the three enhancer elements identified as the Tax-responsive elements with a conserved central core were well conserved

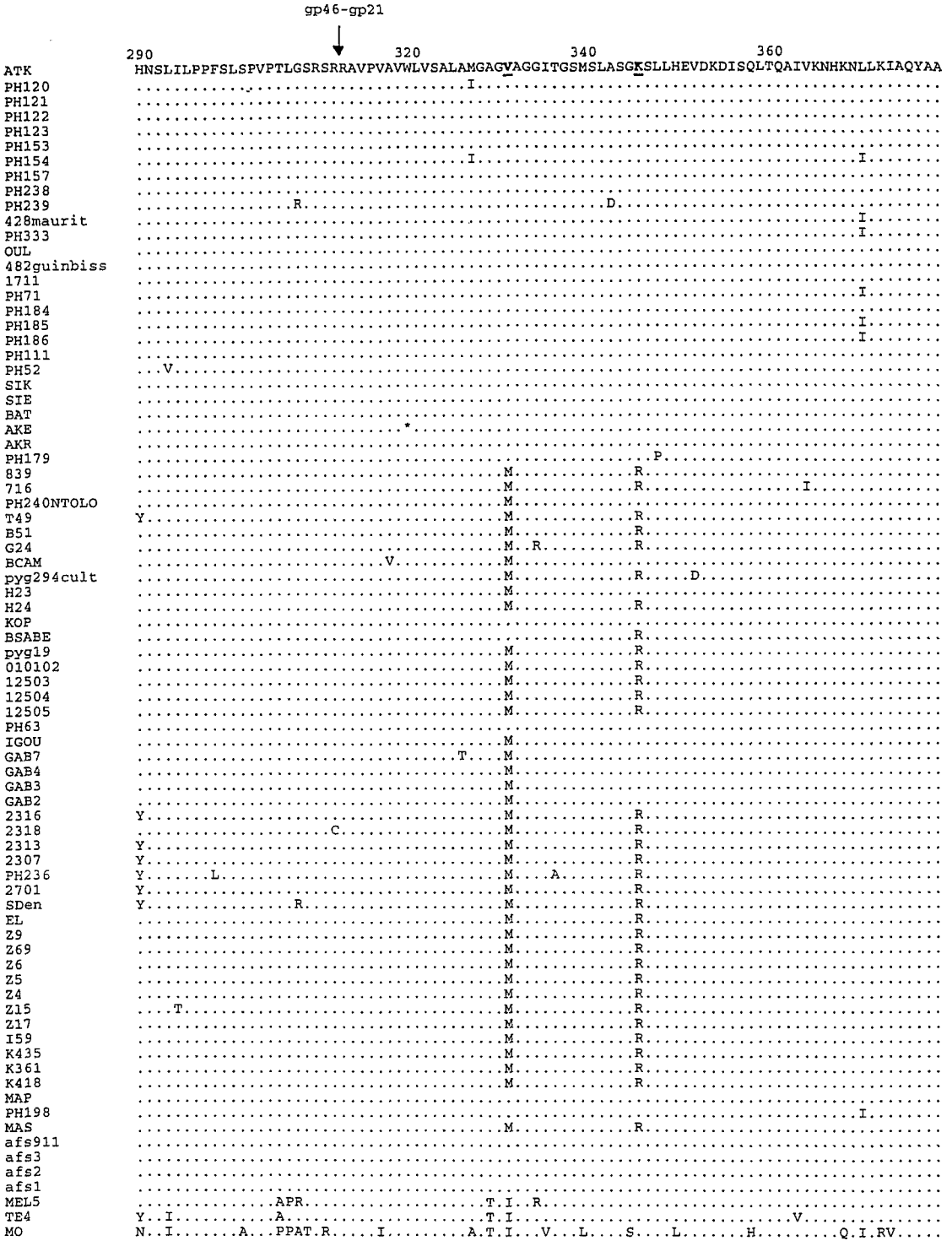

FIG. 1. Putative *env* (5′ end of gp46 and major part of gp21) amino acid alignment of the 58 new African HTLV-1 variant isolates described here compared to 17 previously published African strains and 4 HTLV-1, HTLV-2, and STLV-1 prototypes. The single-letter amino acid code was used. Asterisks indicate translational termination codons. Dots indicate sequence identity with $HTLV\text{-}1_{ATK}$.

(except for a G at position 160 and an A at position 174 in most of the Central African strains and a T in some of them at position 122), as were the c-Ets-responsive elements. In the R region, which contains the Rex-responsive element, a very high degree of conservation was observed in the stem sequence as well as in the Rex binding region (Fig. 2), except for an A at position 523.

(iii) Phylogenetic relationships of the new African HTLV-1 strains with other HTLV-1 and STLV-1 strains. Two portions of the HTLV-1 genome were used for phylogenetic analysis.

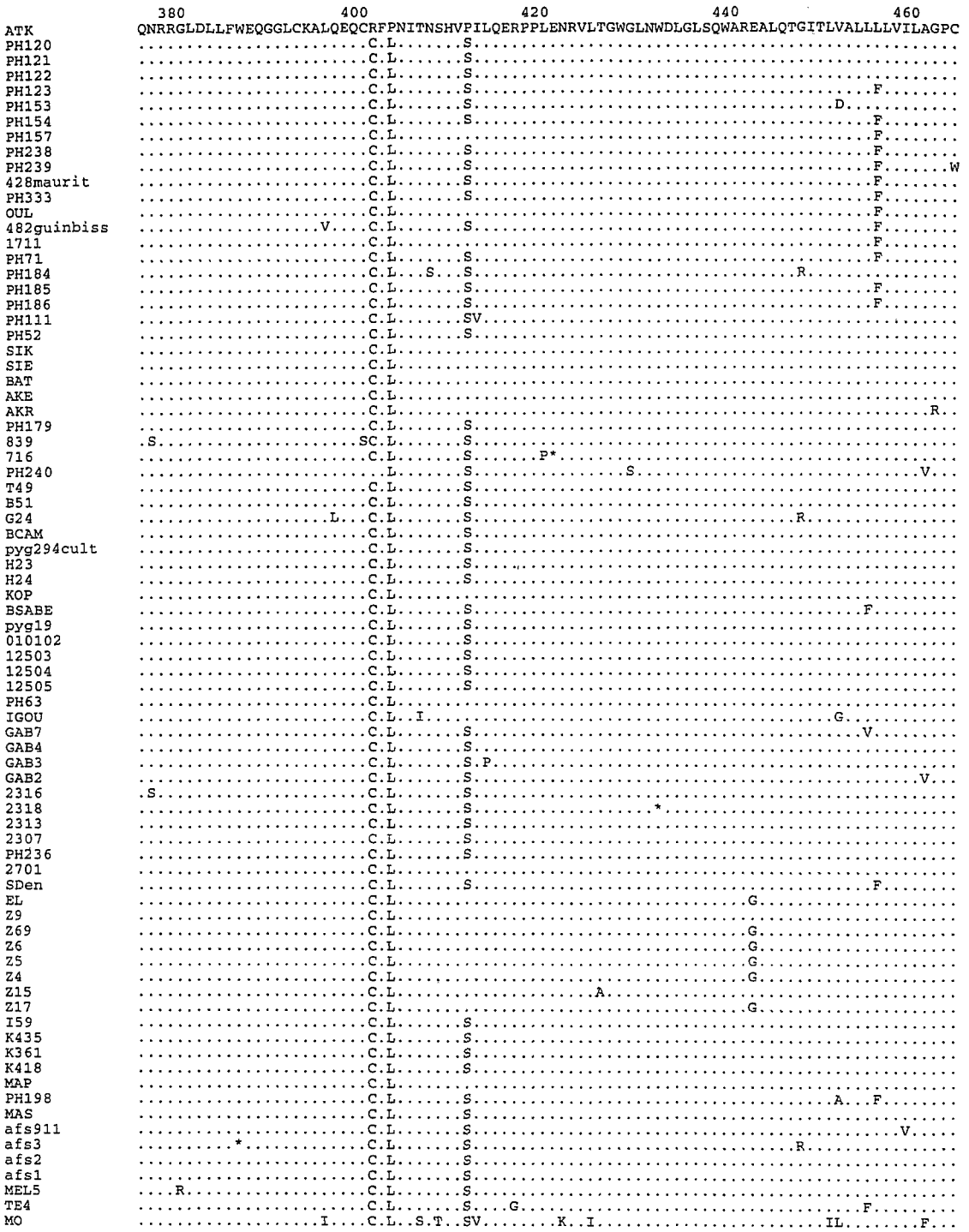

FIG. 1—*Continued.*

The first was a 522-bp fragment of gp21 obtained for all 58 new strains. The second was the complete LTR sequence obtained for nine of our new HTLV-1 strains of African origin. Since tree building algorithms rely on different assumptions, we used two different methods (NJ and DNA maximum parsimony) to increase the reliability of the derived tree topologies. Furthermore, we also used a modified NJ method (59) which is well adapted to closely related sequences (<10% nucleotide sequence difference) such as those encountered with HTLVs.

Comparative analysis of the gp21 *env* gene. Comparative analysis of the gp21 *env* gene was performed with 124 HTLV-1 and STLV-1 sequences, including the 58 new ones, all the other available HTLV-1 (2, 3, 7, 10, 17, 20, 23–25, 31, 37, 48, 50, 52, 60, 63, 68, 69) and STLV-1 (42, 47) sequences of African origin, all the Melanesian HTLV-1 and Asian STLV-1 sequences, and selected sequences from the cosmopolitan subtype, including prototype strains (ATK, MT2, H5, and TSP1). In the first analysis performed with the 93 available HTLV-1

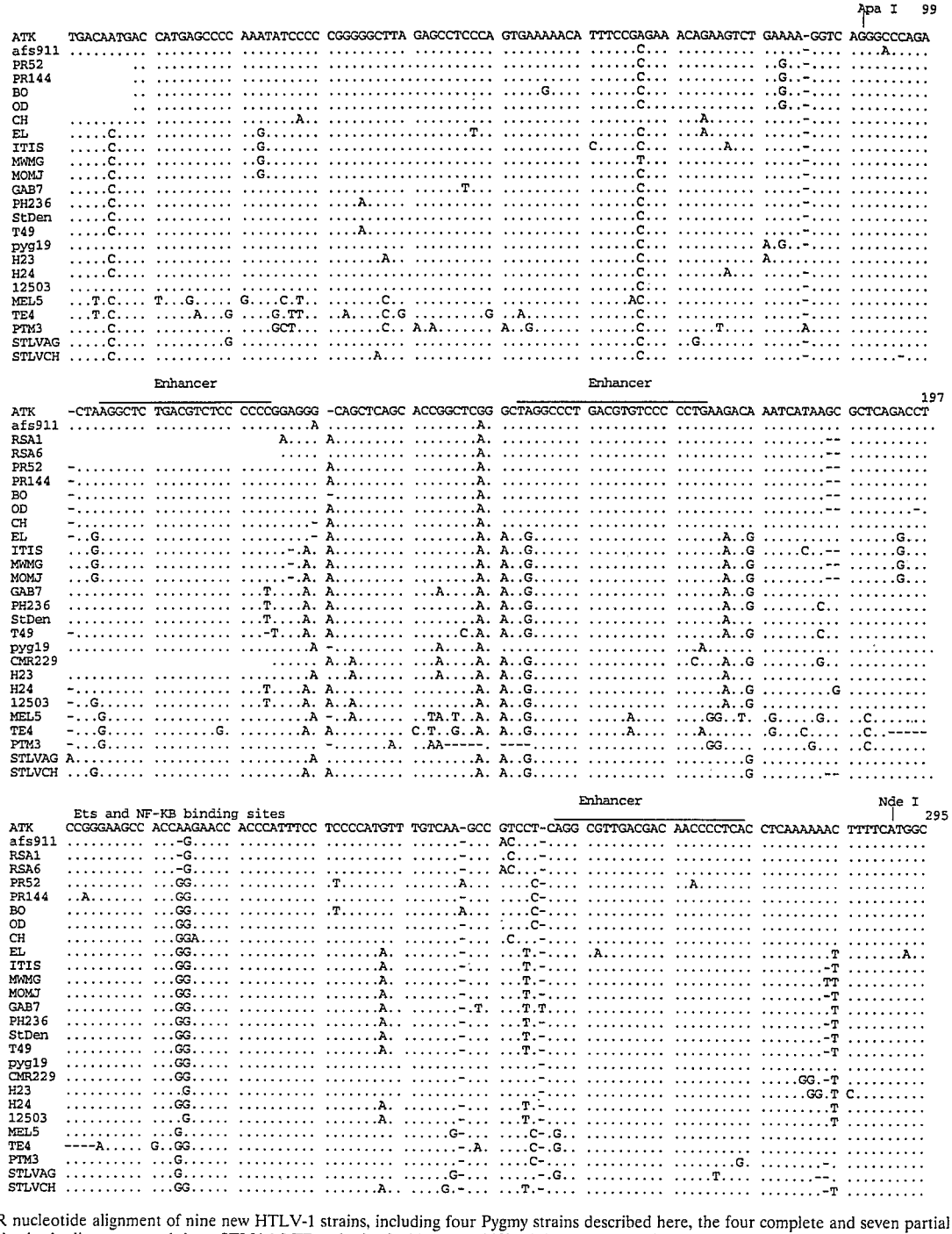

FIG. 2. LTR nucleotide alignment of nine new HTLV-1 strains, including four Pygmy strains described here, the four complete and seven partial LTRs available for African strains in the literature. and three STLV-1 LTRs, obtained with the CLUSTAL W program and minimal manual editing. Dots indicate sequence identity with HTLV-1_{TK}. A dash indicates a deletion. Details about strains are given in Table 1.

sequences (with the STLV-1 PTM3 as an outgroup), four main groups (or clades), including a new molecular subtype, were clearly identified on the basis of consistent topological association in two phylogenetic analyses. As seen in the trees of Fig. 3A and B, obtained by the NJ method with 500 and 100 replicates, respectively, the first clade (bootstrap values of 45% by the NJ method, 44% by the modified NJ method, and 37% by the maximum parsimony method). which included all the new West and South African strains. corresponded to the cosmopolitan subtype (HTLV-1 subtype A). Within this clade, four strains from the Ivory Coast, one from Senegal, one from Burkina Faso, and one from Jamaica (of African ancestry) formed a small separate group (bootstrap values of 65% by the NJ method, 67% by the modified NJ method, and 63% by the maximum parsimony method) with several specific mutations (an A at positions 73, 250, and 457 and a G at position 379). The second major group included the great majority of the new Central African HTLV-1 strains. Within this large group (bootstrap values of 67% by the NJ method, 62% by the modified NJ method, and 37.5% by the maximum parsimony meth-

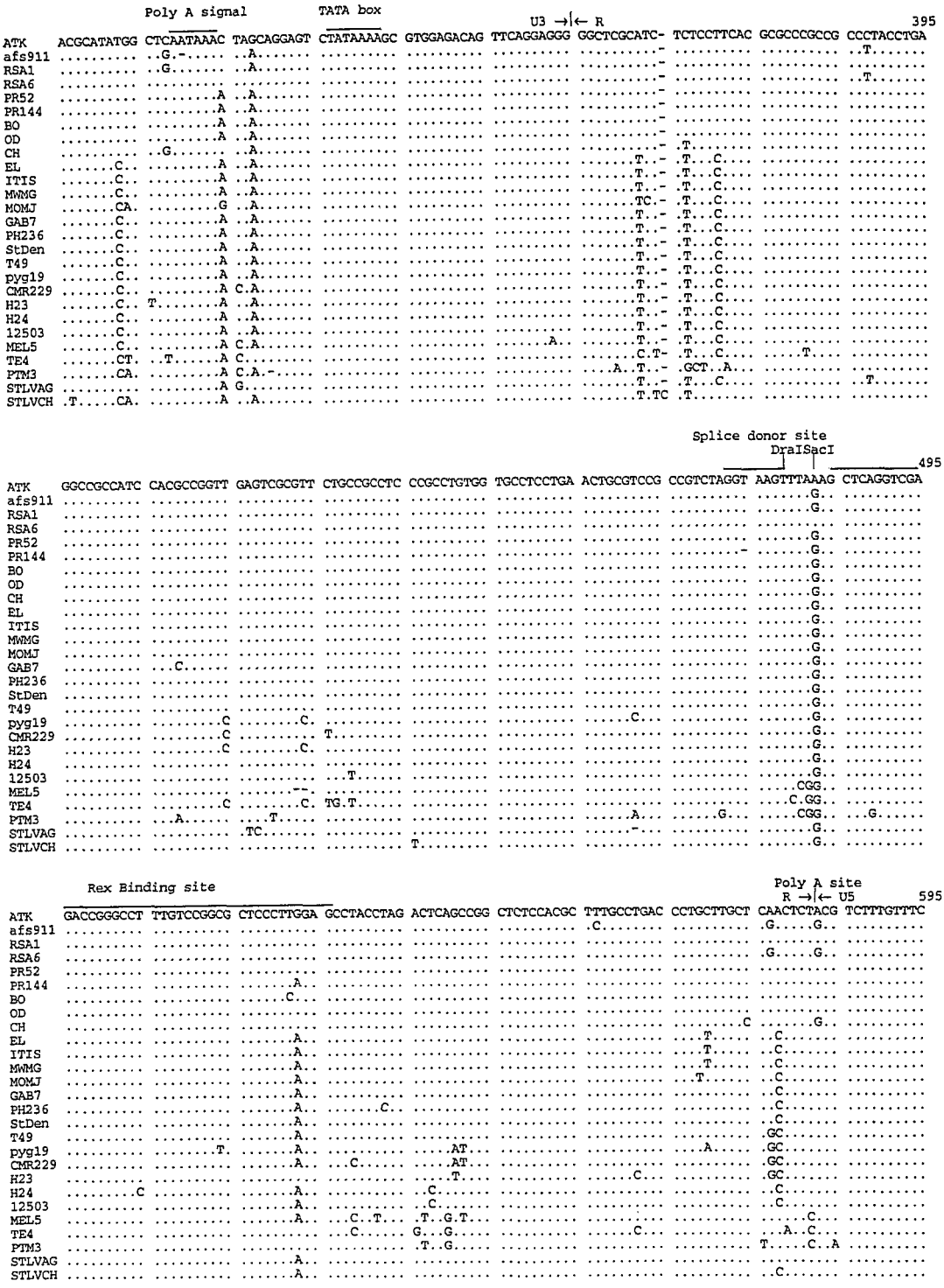

FIG. 2—*Continued.*

od), which included 37 strains, there was evidence of three subclusters corresponding roughly to the geographical origins of samples. Thus, 13 of the 15 Zairian strains clustered together (bootstrap values of 65% by the NJ method, 66% by the modified NJ method, and 46% by the maximum parsimony method) in a group representing an extension of the original HTLV-1 subtype B). The Gabonese and Cameroonian strains, despite being slightly more intermixed, formed two groups with bootstrap values ranging from 57 to 62% by the NJ method. Furthermore, by both methods, all the Pygmy strains, except two, formed a separate group; however, the bootstrap values were low. We propose that the name HTLV-1 subtype B, which was initially given to the EL isolate (60, 63), representing the original Zairian prototype, be extended to this large Central African group.

The third clade, which occupied a unique position between

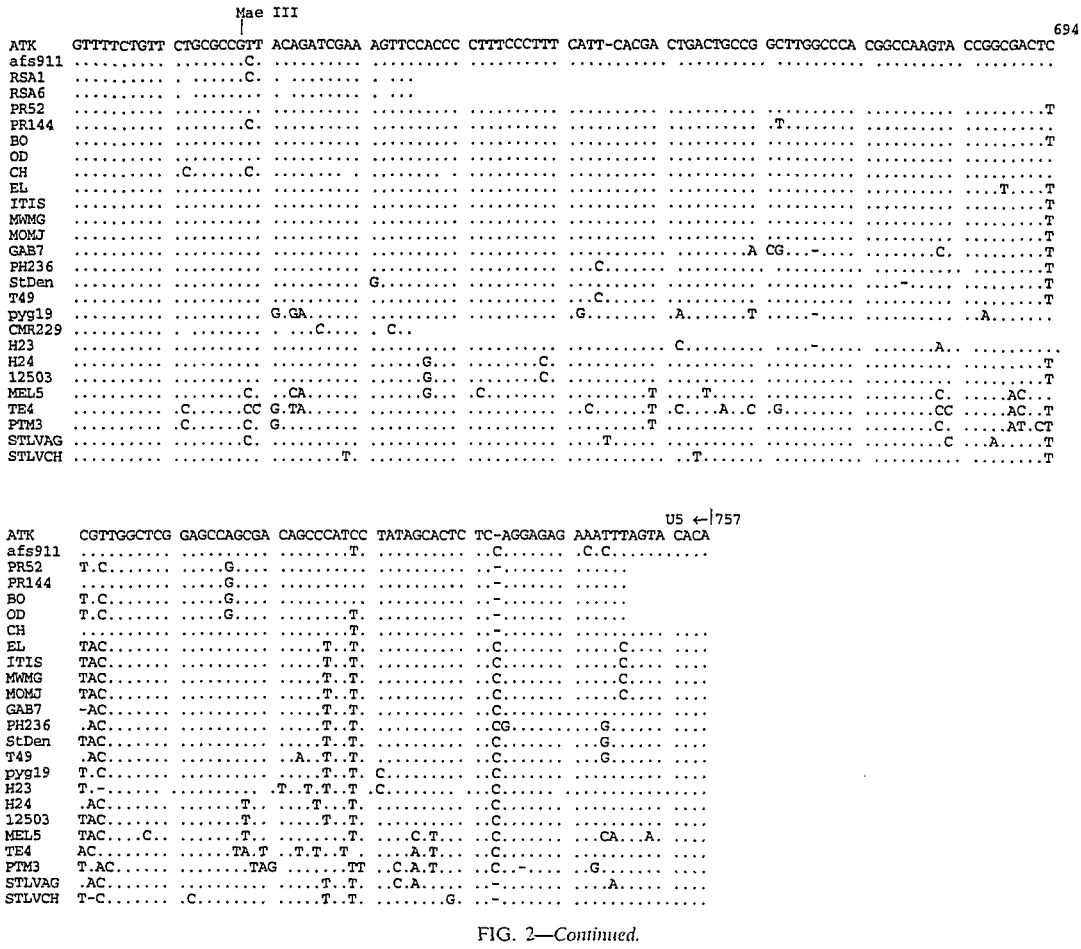

FIG. 2—*Continued.*

the Central African group (HTLV-1 subtype B) and the Melanesian group (HTLV-1 subtype C), appeared as a new, well supported, phylogenetic cluster (bootstrap values of 84% by the NJ method, 81% by the modified NJ method, and 78% by the maximum parsimony method). This new cluster included three of our new Central African strains. Two were from Pygmies, one from southern Cameroon (H23) and one from the CAR (pyg19), and the third was from a non-Pygmy individual living in East Gabon (2318). These three strains shared specific mutations which were present only in this new genotype (a C at position 343 and a T at position 520 for these three strains and a C at position 406 for two of them). We propose that this new molecular group be named HTLV-1 subtype D.

The fourth main clade comprised the Melanesian HTLV-1 strains (those from Papua New Guinea, Solomon Islands, and Australia), which are the most divergent viruses, and was supported by 100% bootstrap values in all analyses.

The BSABE strain from a patient with TSP/HAM living in the eastern part of the CAR occupies a unique position between the large Central African clade and the new molecular subtype (HTLV-1 subtype D) in NJ analysis (Fig. 3A). In maximum parsimony analysis, it occupies a position between the cosmopolitan clade and the Central African one. This new strain may represent the prototype of another HTLV-1 subtype.

The diversity values within and between these four subtypes are shown in Fig. 4A. The corresponding mutation tree (Fig. 4B) shows the most frequent nucleotide positions specific to each subtype and/or subgroup.

In the phylogenetic analysis (based on 124 HTLV-1 and STLV-1 sequences) including all the 31 available STLV-1 *env* sequences (42, 47), the four HTLV-1 geographically related clades were mixed and interspersed between several STLV-1 clades (Fig. 3B). In the NJ analysis, the West African PPA5X28 (47) (an STLV-1 strain from a red baboon) clustered with the cosmopolitan HTLV-1 subtype with a low bootstrap value; however, it preceded the divergence of this group. In all analyses, a clade comprising three chimpanzee strains (PTR114.1, PTR3570, and PTRX43) (47) was included in the Central African HTLV-1 subtype B cluster. Furthermore, it is noteworthy that the variant BSABE strain showed a weak tendency to cluster with three STLV-1 strains in modified NJ and maximum parsimony analyses (not shown). These three *Cercopithecus* strains originated from Uganda, an area not far away from the eastern region of the CAR from which the patient with strain BSABE originated. All the other African STLV-1 strains formed stable clusters which did not intermix with the Central African HTLV-1 strains. In particular, there was no STLV-1 strain related to the new HTLV-1 subtype D, which still formed a well supported phylogenetic clade (74 to 77%) in all analyses.

LTR analysis. For LTR analysis, two phylogenetic studies using either the complete LTR (3, 10, 12, 17, 24, 39, 42, 44, 48, 52, 63, 72, 75, 78, 79, 80) or only a 505-bp LTR segment (ATK positions 144 through 649) (9, 45, 55, 58, 61, 66, 67, 80) and

including 38 and 54 HTLV-1 and STLV-1 strains, respectively, were done. This allowed the inclusion of the 11 African strains for which only partial sequences were available in the literature. In the complete LTR analysis (Fig. 3C), the two phylogenetic methods identified four main well supported genomic clades, including the proposed new HTLV-1 D group. The first clade (bootstrap values of 94% by the NJ method, 88% by the modified NJ method, and 95% by the maximum parsimony method) corresponded to the cosmopolitan group (HTLV-1 subtype A) and included the South African strain afs911. The second group (bootstrap values of 79% by the NJ method, 64% by the modified NJ method, and 48% by the maximum parsimony method) corresponded to the Central African cluster (HTLV-1 subtype B) and included the new Gabonese and Cameroonian strains, the previously known Zairian ones, and the STLV-1 CH isolate from Sierra Leone. Within this group, the two Pygmy strains 12503 and H24 were highly related to each other in all analyses (91 to 95% bootstrap values). STLV-1 AG was related to this Central African cluster but was located at a position between the latter group and the Melanesian clade. The third group, corresponding to the two new Pygmy strains H23 and pyg19, formed a well supported and distinct new molecular cluster group (HTLV-1 subtype D) (bootstrap values of 99% by the NJ method, 95% by the modified NJ method, and 94% by the maximum parsimony method) and was located between the cosmopolitan and Central African clades. The fourth group correspond to the more distant Asian HTLV-1 and STLV-1 strains and included Mel 5 (Solomon Islands) and the STLV-1 strains PTM3 and TE4 (Indonesia [Sulawesi]). The results of phylogenetic analyses performed on the 55 partial (505-bp) LTR sequences (using HTLV-2 MO as an outgroup [71]) were very similar to those of analyses using complete LTR sequences. However, the bootstrap values of main clades were always lower. Within the cosmopolitan clade, at least the following two subgroups were identified with low bootstrap values: one corresponding to the Northern African strains (21) and the other corresponding to some strains from the Ivory Coast and Ghana (45, 55). These two subgroups correspond to subtypes D and C, respectively, in the classification of Miura et al. (55).

Based on sequence comparisons and phylogenetic analyses of both the gp21 *env* gene and complete LTR, a new classification of HTLV-1 genotypes is proposed in Table 3. Each of the four subtypes (HTLV-1 A to D) is well supported phylogenetically (high bootstrap values), but within these subtypes exist subgroups characterized by specific mutations.

TABLE 3. New classification of HTLV-1 genotypes in four major subtypes[a]

Genetic subtype	Geographical subtype[b]	Nucleotide divergence (%)[c]	
		LTR	*env* (gp21)
A	Cosmopolitan Western African countries (++) (Senegal, Mauritania, Mali, Guinea-Bissau, Burkina Faso, Ivory Coast) Central African countries South Africa (++) America (North, Central, South) (++) Caribbean (++) Iran, Iraq (++) Japan (South) Japanese Japan (++) India	0–2 1.3–2.9	0–2.5 0.9–2.7
	West African Ivory Coast, Ghana, Senegal Caribbean North African Algeria, Morocco (++)	2.6–3.4	1.1–4
B	Central African Zaire, Gabon (++) Cameroon, CAR (++)	4.1–6	2.1–4.2
D	New Central African Cameroon, CAR, Gabon	4.9–6.8	2.1–4.6
C	Australo-Melanesian Papua New Guinea (++) Solomon Islands (++) Australia (++)	8.8–9.3	6.5–8.2

[a] Based on sequence comparisons and phylogenetic analyses of both the gp21 *env* gene and the complete LTR. Each of the four subtypes (HTLV-1 A through D) is well supported phylogenetically, but within these subtypes there exist subgroups characterized by specific mutations.
[b] ++, the corresponding subtype is commonly found in the given area.
[c] Compared with the cosmopolitan group.

DISCUSSION

This study was the first comprehensive study of HTLV-1 strains from the African continent; it included investigations of 58 new strains originating from 13 countries comprising most of the regions where this oncoretrovirus is highly endemic. It led to three new findings.

The major finding was the description of a new genomic subtype of HTLV-1. This new, phylogenetically well supported genotype, which we propose to name HTLV-1 subtype D, was found only in three individuals from Central Africa. The identification of a separate genotype was strongly validated by the finding of high bootstrap values in the two phylogenetic analyses performed on both the gp21 *env* gene and on the LTR region. This conclusion is also supported by the fact that these individuals lived in widely separated areas of the western part of Central Africa without any evidence of recent contact. Recent studies (28, 42, 47, 48, 65, 80) have suggested that the present world repartition of HTLV-1 genotypes is the result of at least three independent events, interspecies transmission of STLV-1 to humans (as in the case of chimpanzees in Central Africa), independent evolution of the virus in some remote areas (as in Melanesia), and migrations of infected populations (as, for example, during the slave trade). We therefore hypothesize that the presently described new HTLV-1 genotype (subtype D) may have taken its origin in interspecies transmission from monkeys to humans in Central Africa and that there exists or existed in Central Africa an STLV-1 closely related to this HTLV-1 subtype D genotype. The presence of this new genotype among two Pygmies, whose culture includes hunting with many occasions for contact with monkey blood, reinforces the possibility of such a method of viral acquisition. Studies are ongoing in Gabon, the CAR, and Cameroon to search for an HTLV-1 subtype D-related STLV-1 strain in several monkey species. Whether some of the recently reported HTLV-1 variant strains from Gabon (sequences available only for the gp46 *env* gene) (56) are related to this new molecular genotype remains to be elucidated by further sequencing studies. Furthermore, preliminary analysis based on the only available partial LTR sequence seems to indicate that another recently described isolate from a Pygmy living in southern Cameroon

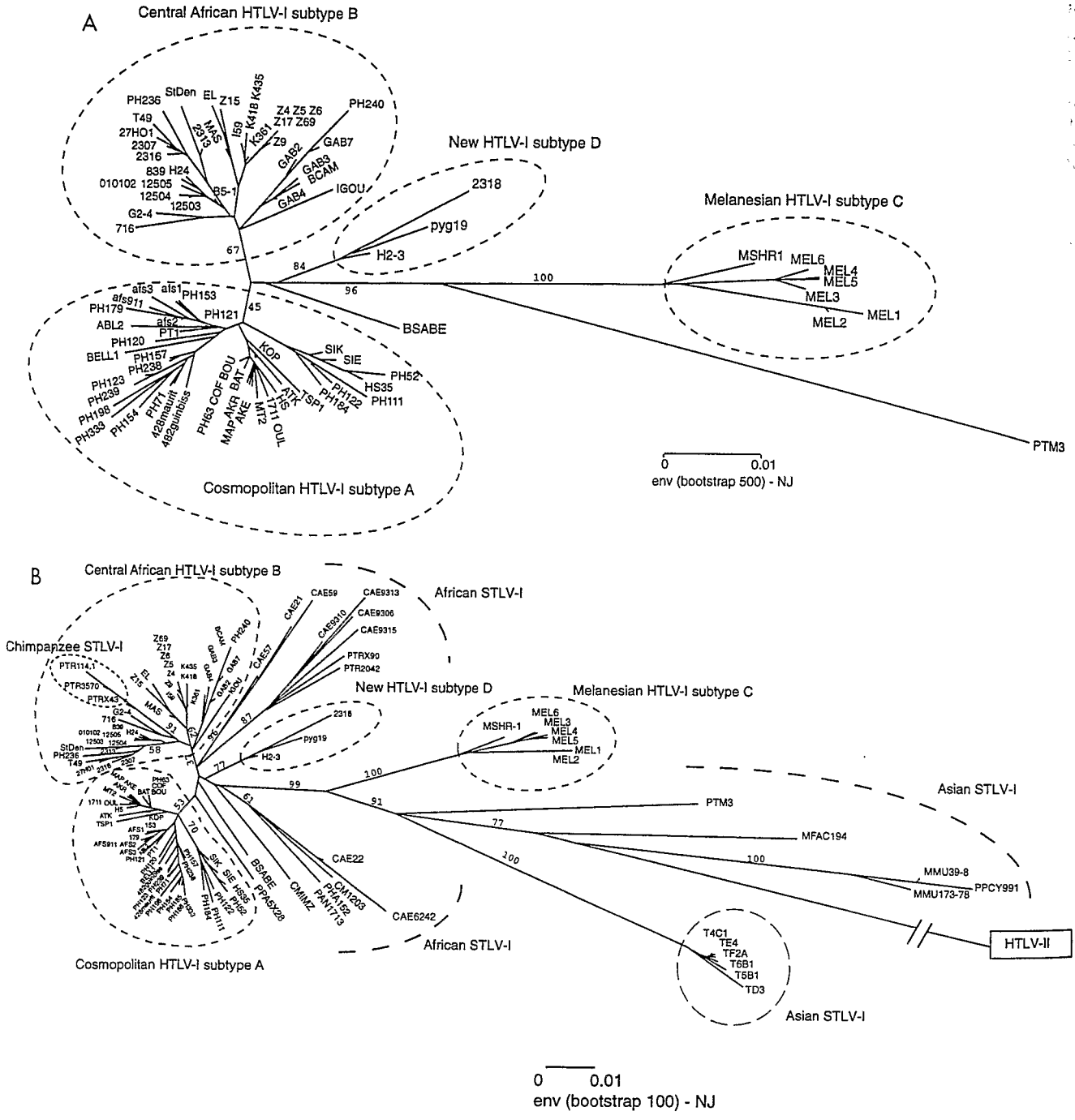

FIG. 3. Phylogenetic trees. (A) Phylogenetic tree constructed by the NJ method using the NEIGHBOR program with a fragment of 522 bp encompassing most of gp21 and the carboxy terminus of gp46 for 93 HTLV-1 isolates, including all 75 African ones. PTM3 (STLV-1 isolate) was used to root the tree. (B) Phylogenetic tree constructed by the NJ method with a fragment of 522 bp of the *env* gene for 124 isolates, including the 36 available STLV-1 strains. The HTLV-2 MO isolate (71) was used as an outgroup to root the tree. (C) Phylogenetic tree constructed by the NJ method using the MEGA program with the complete LTR (755 bp). The numbers at some nodes (bootstrap values) indicate frequencies of occurrence for 100 (B) or 500 (A and C) trees.

(9) may be related to the HTLV-1 subtype D group. The absence of detection of HTLV-1 antigens in PBMC cultures and the lack of isolation of HTLV-1 from the PBMCs of the two Pygmies (pyg19 and H23) infected with this new molecular genotype (HTLV-1 subtype D) were possibly related to the low HTLV-1 load present in their PBMCs, as evidenced by the very faint bands detected by PCR with different sets of primers and indirectly suggested by their very low HTLV-1 antibody titers (1/20 and 1/40). In contrast, detection of viral antigens in PBMC cultures was more consistent, establishment of cell lines was easier, and antibody titers were higher with most of the other Pygmy strains belonging to HTLV-1 subtype B (Central African). Thus, such low viral loads might be specifically related to some HTLV-1 strains, including the new HTLV-1 subtype D genotypes.

The second new finding was the demonstration, based on the

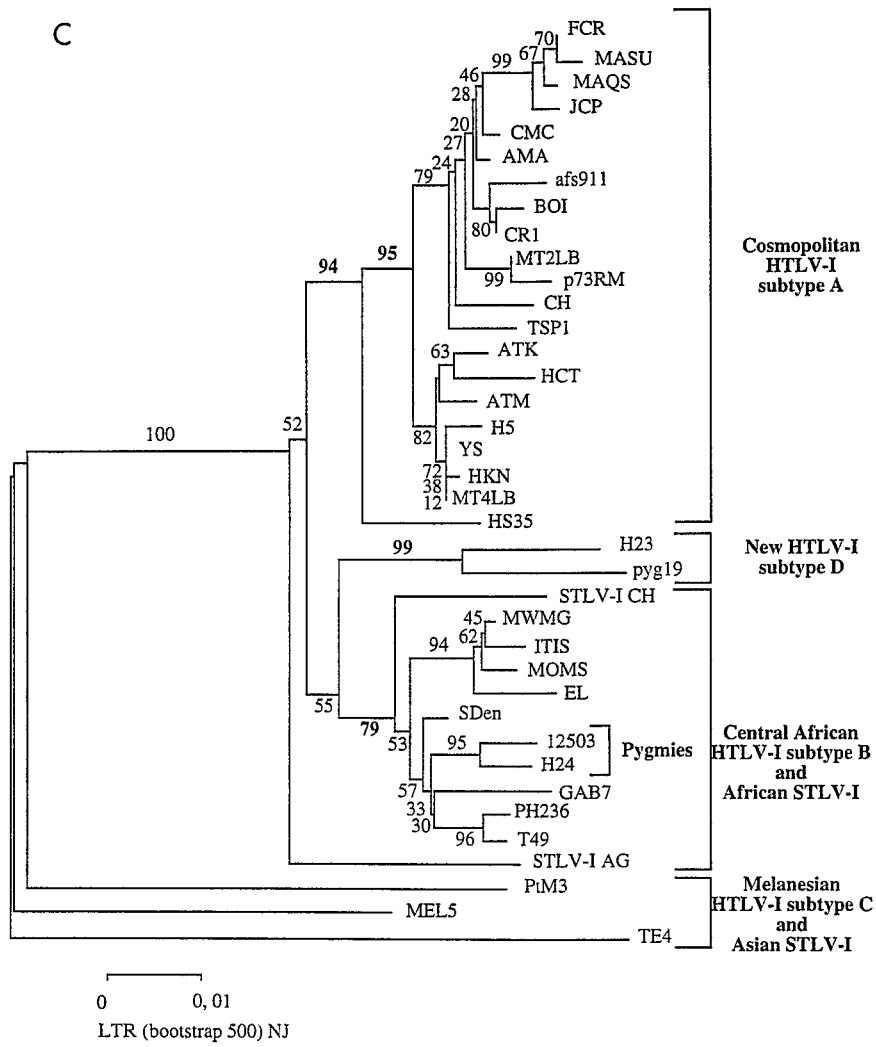

FIG. 3—*Continued.*

study of 36 new HTLV-1 strains, of greater HTLV-1 genomic diversity in strains from Central Africa than originally thought. Furthermore, within this large Central African clade, there clearly exist subgroups specific to the geographical origins of strains. These groups, which differ by 1.2 to 3.5% in nucleotide sequence, are not always highly supported phylogenetically but are characterized by specific mutations, as illustrated by the Zairian strains. The following two main and nonexclusive hypotheses can be proposed to explain such geographically restricted genetic diversity: (i) an ancient episode of STLV-1 transmission to a human group followed by a long period of independent evolution which occurred simultaneously in several separated and isolated human populations, with possibly different rates of genetic variability, or (ii) several more recent episodes of interspecies viral transmission from simian reservoirs, leading in each human recipient group to a different genetic strain. We favor the latter hypothesis.

Of interest was the fact that one of the Central African HTLV-1 subtype B subgroups was comprised predominantly of Pygmy strains. All the Pygmies of this subgroup (except pyg294cult of the Bakola tribe) were of the same original ethnic origin (Baka and Nzakara) despite living currently in two areas 800 km apart (1). Pygmies are considered the oldest inhabitants of Central Africa, and genetic studies have indicated that differences between Pygmy populations and surrounding agricultural people are great enough to have required about 20,000 years of isolation (1). Furthermore, as stressed by Goubau et al., it is very unlikely that the HTLV-1 strains present in Pygmies would represent a recent importation into these groups from other neighboring human populations (33, 34).

The very close relationships (>98% nucleotide similarity) among some STLV-1 strains from chimpanzees and HTLV-1 subtype B strains present in Pygmies and other Central African inhabitants reinforce the possibility of relatively recent interspecies transmission in this forest area (47, 65). Thus, an important new finding of our study was that there exist at least two different HTLV-1 genotypes in Pygmies currently living in the CAR and Cameroon. This is well illustrated by the presence of these two variants in a couple of Pygmies living in southern Cameroon.

Finally, this study demonstrated that all 23 HTLV-1 strains from the western part of Africa (including 18 new strains described here) belong to the cosmopolitan genotype (subtype A), with some of them constituting a small separate cluster within the large cosmopolitan clade. These 23 viruses origi-

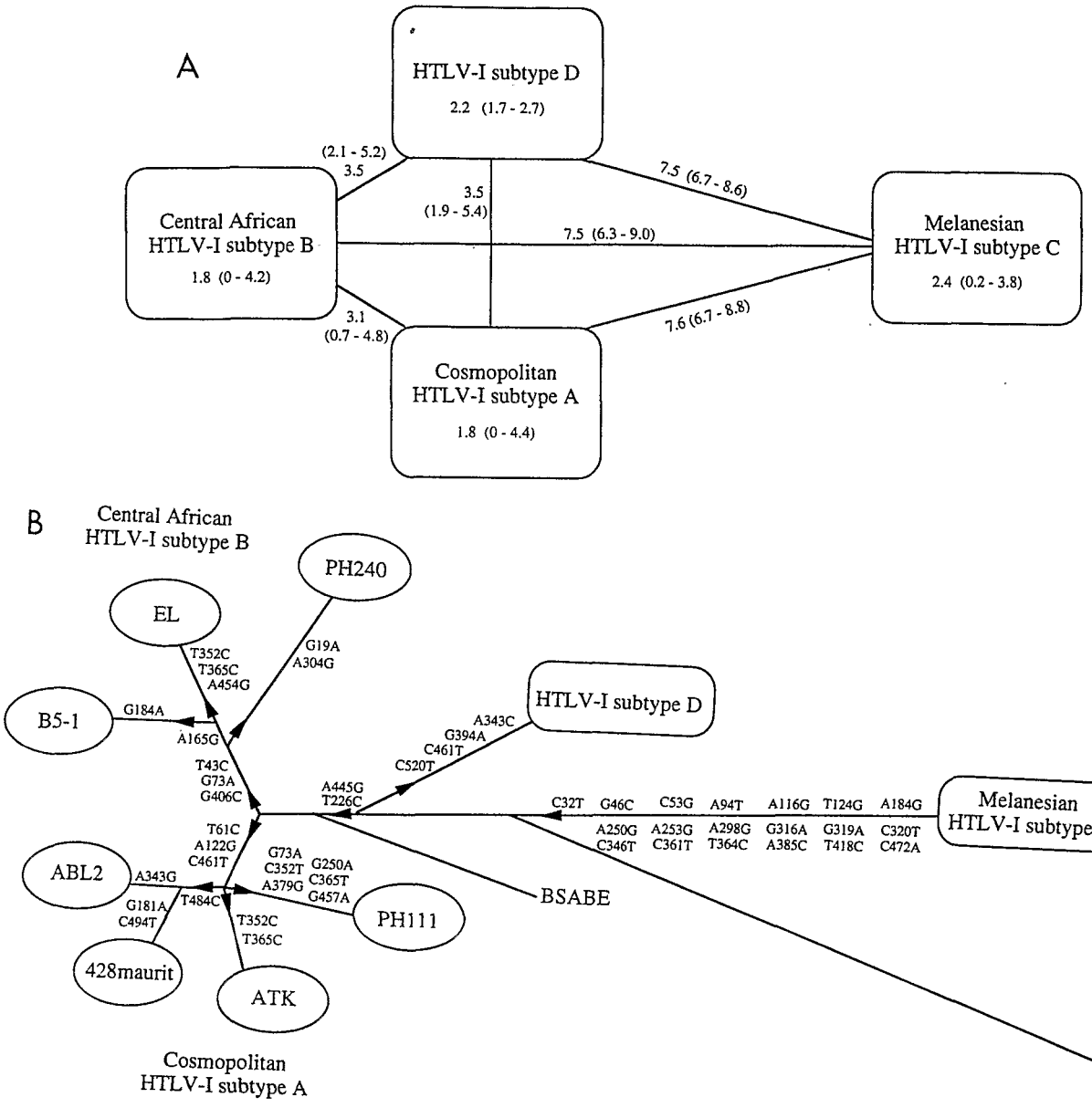

FIG. 4. (A) Inter- and intrasubtype nucleotide variability. The average within-subtype variabilities are noted in the boxes (the minimum and maximum values are given parenthetically). The between-subtype variabilities are associated with the corresponding branches. The lengths of branches between subtypes do not reflect distances. Each value is the percentage of mismatches between sequences. (B) Mutation tree. Subgroups and their corresponding branches were derived from the HTLV-1 phylogenetic tree. Subgroups defined from the HTLV-1 subtypes A and B are labelled with one of their sequences. An arbitrary direction is given to each branch, with the general direction going from HTLV-1 subtype C and diverging to subgroups after the central node. The nucleotide mutations with their positions in the multiple alignment are associated with their corresponding branches. For example, HTLV-1 subtype D differs from the other groups by a C at position 343 rather than an A. A subgroup of HTLV-1 subtype A, labelled by the reference sequence ABL2, differs by the mutation of A to G at the same position. In the Melanesian subtype, a C at position 32 differs from a T in all the other sequences.

nated from seven countries (Senegal, Mauritania, Cape Verde, Guinea-Bissau, Mali, Burkina Faso, and Ivory Coast), most of which were major centers for the slave trade from the 16th century to 19th century. Of importance, these African HTLV-1 strains are identical or very closely related to the HTLV-1 strains present in the Americas (North, Central, Caribbean, and South), which all belong to the cosmopolitan genotype, as demonstrated by several groups (3, 17, 18, 25, 27, 45, 52, 55, 61, 68, 73). Therefore, our view, based mostly on these new findings but also on historical background, of the clustering in the Americas of HTLV-1 strains mainly in populations of African ancestry (5, 6, 57) and of its rarety in pure isolated Amerindian populations without contacts with Afro-Americans is that the great majority of the HTLV-1 strains found in the Americas are of recent African origin (from the 16th to 19th centuries) and not of Asian origin, as is still supported by others (55, 82). Exceptions to this include, of course, recent Japanese immigrants to South America and perhaps some British Columbian Indians (61). Such views, already suggested by us and others (25, 27, 48, 67, 73, 76, 78) for smaller series of African strains, contrast strongly with the hypothesis of another group (55) which is still convinced (by studying only part of the LTR

region) that the majority of HTLV-1 strains from the American continents originated from ancestral Mongoloids who migrated from Asia to the American continents. Moreover, the data obtained in the present study for HTLV-1 strains from South Africa confirm and extend the contention that HTLV-1 strains from this region belong to the cosmopolitan group (82).

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