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Isolation and Characterization of a New Simian T-Cell Leukemia Virus Type 1 from Naturally Infected Celebes Macaques (*Macaca tonkeana*): Complete Nucleotide Sequence and Phylogenetic Relationship with the Australo-Melanesian Human T-Cell Leukemia Virus Type 1

FERA IBRAHIM, GUY DE THÉ, AND ANTOINE GESSAIN*

Unité d'Epidémiologie des Virus Oncogènes, Institut Pasteur, 75728 Paris Cedex 15, France

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A study of simian T-cell leukemia virus type 1 (STLV-1) infection in a captive colony of 23 *Macaca tonkeana* macaques indicated that 17 animals had high human T-cell leukemia virus type 1 (HTLV-1) antibody titers. Genealogical analysis suggested mainly a mother-to-offspring transmission of this STLV-1. Three long-term T-cell lines, established from peripheral blood mononuclear cell cultures from three STLV-1-seropositive monkeys, produced HTLV-1 Gag and Env antigens and retroviral particles. The first complete nucleotide sequence of an STLV-1 (9,025 bp), obtained for one of these isolates, indicated an overall genetic organization similar to that of HTLV-1 but with a nucleotide variability for the structural genes ranging from 7.8 to 13.1% compared with the HTLV-1 ATK and STLV-1 PTM3 Asian prototypes. The Tax and Rex regulatory proteins were well conserved, while the pX region, known to encode new proteins in HTLV-1 (open reading frames I and II), was more divergent than that in the ATK strain. Furthermore, a fragment of 522 bp of the gp21 *env* gene from uncultured peripheral blood mononuclear cell DNAs from five of the STLV-1-infected monkeys was sequenced. Phylogenetic trees constructed with the long terminal repeat and *env* (gp46 and gp21) regions demonstrated that this new STLV-1 occupies a unique position within the Asian STLV-1 and HTLV-1 isolates, being, by most analyses, related more to the Australo-Melanesian HTLV-1 topotype than to any other Asian STLV-1. These data raise new hypotheses on the possible interspecies viral transmission between monkeys carrying STLV-1 and early Australoid settlers, ancestors of the present day Australo-Melanesian inhabitants, during their migrations from the Southeast Asian land mass to the greater Australian continent.

The primate T-lymphotropic viruses (PTLV), which include human T-cell leukemia virus type 1 (HTLV-1) (62), simian T-cell leukemia virus type 1 (STLV-1) (54), HTLV-2 (36), STLV-2 (7), and the recently isolated PTLV-L (24) and STLV-pan p (23, 47), constitute a group of highly related human and simian retroviruses sharing common biological, immunological, and molecular features. HTLV-1 is the etiological agent of a malignant CD4 lymphoproliferation (adult T-cell leukemia) (30, 77) and of a chronic progressive neuromyelopathy (tropical spastic paraparesis/HTLV-1-associated myelopathy [TSP/HAM]) (16). Furthermore, it has been associated with cases of uveitis, arthritis, polymyositis, and infective dermatitis. In STLV-1-infected monkeys, such as African green monkeys, baboons, and gorillas, cases of adult T-cell leukemia-like diseases (3, 31, 51, 59, 66, 69, 76, 78, 80) have been observed, while no neurological diseases similar to TSP/HAM in such animals have been reported to date.

Seroepidemiological studies have shown the presence of STLV-1 antibodies in a large variety of Old World monkeys and apes (2, 5, 9–12, 15, 26, 28, 32–34, 39, 46, 48, 52–55, 67, 83–88), including chimpanzees (*Pan troglodytes*), gorillas (*Gorilla gorilla*), grivet monkeys (*Cercopithecus aethiops aethiops*), baboons (*Papio anubis*), cynomolgus or crab-eating macaques (*Macaca fascicularis*), pig-tailed macaques (*Macaca nemestrina*), stump tailed macaques (*Macaca arctoides*), rhesus macaques (*Macaca mulatta*), bonnet macaques (*Macaca radiata*), lion-tailed macaques (*Macaca silenus*), toque monkeys (*Macaca sinica*), and Celebes macaques (*Macaca nigrescens*, *Macaca nigra*, *Macaca hecki*, *Macaca tonkeana*, *Macaca maura*, *Macaca ochreata*, and *Macaca brunnescens*), but not in New World monkeys (28, 32, 37) or in Prosimians (references 28 and 32 and unpublished data). Molecular epidemiological studies of HTLV-1 have clearly demonstrated that the low genetic variability of HTLV-1 in vivo depends more upon geography than upon pathologies (20, 38, 60, 63, 64, 70, 72) and can be used as a molecular means to follow the migration of infected populations in both the distant and recent past (20, 89). On the basis of sequence analysis (of the long terminal repeat [LTR], *pol*, and *env*) and studies of the LTR by restriction fragment length polymorphism analysis (38, 81), there are five different specific HTLV-1 geographical genotypes (or subtypes), i.e., the Cosmopolitan, the Japanese (closely related to the Cosmopolitan), the West African, the Central African, and the Australo-Melanesian subtypes. The last of these, which is the most divergent (1, 18, 22, 72, 88–91), differs from the other subtypes by around 7% nucleotidic substitution and consists of at least three subgroups: one from Papua New Guinea, one from the Solomon islands, and one from Australia. These Australo-Melanesian HTLV-1 strains occupy a unique phylogenetic position between Asian STLV-1 strains and all of the other less-divergent HTLV-1 subtypes (Cosmopolitan, Japanese, and African).

Two recent reports (40, 67) based on phylogenetic analyses of the *env* and *pol* genes of HTLV-1 and STLV-1 from Central and West Africa have provided clear evidence that multiple,

* Corresponding author. Mailing address: Unité d'Epidémiologie des Virus Oncogènes, Institut Pasteur, 28, rue du Dr Roux, 75728 Paris Cedex 15, France. Phone: (33) 1 45 68 89 30. Fax: (33) 1 45 68 89 31.

continued interspecies transmission of HTLV-1 and STLV-1 between primates, including humans, has occurred in the distant and recent past in these areas. One can hypothesize that similar interspecies virus transmission may have taken place in Asia and that STLV-1 strains genetically related to the Australo-Melanesian strains of HTLV-1 may exist in populations of nonhuman primates presently inhabiting islands that served as the migratory pathways or routes for the early settlers of Papua New Guinea and Australia 30,000 to 40,000 years ago (18, 19, 22, 63, 87, 89). However, despite the wide geographical spread and the large diversity of STLV-1-infected monkeys in Asia (89), only four partial STLV-1 sequences from Southern Asia and Indonesia are available; their exact locations are not defined. Two sequences are from Indonesia (PTM3 from a pig tail monkey [*M. nemestrina*] [85] and C194 from a crab-eating macaque [*M. fascicularis*] [40]), and two are from India (MMU39-83 and MMU173-78 from rhesus macaques [*M. mulata*] [40]).

We report here the isolation of a new STLV-1 originating from a troop of Celebes macaques (*M. tonkeana*), with the first complete nucleotide sequence of an STLV-1 together with seroepidemiological data. *M. tonkeana* originally lived on Sulawesi Island, which is one of the nearest natural monkey habitats to Papua New Guinea (an area devoid of monkeys), but it is also found on one of the two suspected migratory pathways taken by the early Australoid migrants.

MATERIALS AND METHODS

Animals. All animals were kept at the Strasbourg Primatology Center (access supplied by Nicolas Herrenschmidt and Eric André). There were 15 females and 8 males. The troop derived from three founders (RM, DOM, and T2) that originated from the central part of Sulawesi (Celebes Island), Indonesia, and were brought from that area in August 1972. Since then, the entire troop has been maintained isolated in this primatology center for ethological research, without contact with any other monkey species. The animals were never used for any experimental study or infected with any biological material.

Serological assays. All of the serum or plasma samples were tested for HTLV antibodies by three different screening assays: an enzyme-linked immunosorbent assay (ELISA) (Diagnostic Biotechnology, Singapore, Singapore) which contains disrupted HTLV-1 and two recombinant peptides, one specific for HTLV-1 gp46 (amino acids 162 to 209) (MTA1) and the second specific for HTLV-2 gp46 (amino acids 162 to 205) (K55); a particle agglutination (PA) assay (Fujirebio Inc., Tokyo, Japan); and an immunofluorescence (IF) assay with an HTLV-1 (MT2)- or HTLV-2 (C19)-producing cell line. Furthermore, all samples were tested by an HTLV-1/2 confirmatory assay: a Western blot (immunoblot) (WB) which contains disrupted HTLV-1, a recombinant protein (rgp21) which reacts with both HTLV-1 and HTLV-2 antibodies, and the two gp46 peptides MTA1 and K55 (6, 27). The HTLV antibody titers were determined by serial dilution of the plasma samples or the sera by using the PA and IF assays.

Cell culture. Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood (drawn in June 1992 and April 1993) with Ficoll-Hypaque. Ten million cells were placed in a 50-ml culture flask containing 5 ml of RPMI 1640 with 20% heat inactivated fetal calf serum, 1% L-glutamine, and 1% penicillin-streptomycin. During the first 3 days, the cells were stimulated with phytohemagglutinin (Difco) at 2 μg/10^6 cells. The cells were then cultivated in a humidified 5% CO_2–air atmosphere in the same medium as described above plus 10% interleukin-2 (Boehringer, Mannheim, Germany) with biweekly changes. Cells from STLV-1-seropositive animals were also cocultivated with PBMCs from healthy HTLV-1-seronegative blood donors after 8 weeks of culture alone.

Immunovirological studies. An indirect IF assay was performed on the cultured cells to detect viral antigen expression; the assay used either mouse monoclonal antibodies directed against HTLV-1 p19 and p24 (Cambridge Biotech, Wilmington, Del.) or polyclonal sera from HTLV-1-infected individuals and STLV-1-infected monkeys, including the serum of the animal being studied. Cytofluorography analysis of the cultured cells was performed with different monoclonal antibodies directed against human T-cell and B-cell antigens as previously described (49). The production of the p24 core antigen in the culture supernatant was measured by a capture ELISA test that detects HTLV-1, STLV-1, and HTLV-2 p24 (Kit HTLV Ag Assay [Coulter]).

DNA isolation and molecular studies. High-molecular-weight DNA was extracted from the cell lines and digested with *Eco*RI restriction enzyme, and the restricted fragments were separated by electrophoresis, transferred to nylon filters, and hybridized under high-stringency conditions with the PMT-2-3 subcloned *Pst*I fragment of PMT-2 clone or the 1711 total HTLV-1 genomic probe (kindly provided by G. Franchini, National Institutes of Health, Bethesda, Md.).

TABLE 1. Serological findings for 23 *M. tonkeana* monkeys by different HTLV-1 screening and confirmatory assays

Monkey	Age (yr)/sex[a]	ELISA result[b]	PA titer[c]	IF titer[d] with:		WB pattern[e]
				MT2 cells	C19 cells	
RM	32/F	+	2,048	1,280	20	+
T5	11/F	+	8,192	2,560	160	+
TC2	5/M	+	256	640	160	+
TD2	4/M	+	4,096	2,560	640	+
TE3	4/F	+	2,046	1,280	80	+
T4	13/F	+	32,768	5,120	640	+
T6	11/F	+	16,384	1,280	320	+
TD3	4/F	+	16,384	5,120	320	+
T10	18/M	+	128	80	<20	+
T12	?/M	+	4,096	2,560	640	+
TF2	2/F	+	1,024	1,280	80	+
TG1	1/M	+	1,024	1,280	160	+
T7	14/F	+	512	320	160	+
T2	25/F	+	512	640	80	+[f]
TE4	3/F	+	8,192	1,280	160	+[f]
T9	8/M	+	512	80	20	+[f]
TC1	4/F	+	8,192	320	20	+[f]
TG2	1/F	+	64	80	40	I[g]
T1	18/M	−	<16	<20	<20	NT[h]
TA1	8/M	−	<16	<20	<20	NT
TD1	5/F	−	<16	<20	<20	NT
T3	9/F	−	<16	<20	<20	NT
TE2	4/F	−	<16	<20	<20	NT

[a] F, female; M, male.
[b] Obtained with a Diagnostic Biotechnology ELISA HTLV-1 kit.
[c] Reciprocal of last dilution showing positive in the Serodia HTLV-I PA test (>16 was a positive result).
[d] Reciprocal of last dilution showing positive IF on acetone-fixed MT2-CEM and C19-CEM cells (>20 was a positive result).
[e] Obtained with the Diagnostic Biotechnology HTLV blot 2-3.
[f] Reactivity to gag and rgp21 bands, without reactivity to the MTA1 gp46 HTLV-1 peptide.
[g] I, indeterminate (reactivity only to p19).
[h] NT, not tested.

(i) PCR. The PCR amplification of ex vivo PBMC DNAs from STLV-1 antibody-positive animals was accomplished with a seminested PCR using as the outer primer set env 1 and env 22 and as the inner set env 1 and env 2 primers containing the *Not*I and the *Eco*RI restriction sites, respectively. These latter primers amplify a 522-bp envelope fragment (nucleotides 6046 to 6567 of the Japanese prototype HTLV-1 ATK [71]) (equivalent to European Molecular Biology Laboratory bases 6068 to 6589) spanning the carboxy terminus of gp46 and almost the entire transmembrane protein gp21 (20, 40). The PCR was performed in a volume of 100 μl with 1× PCR buffer (10 mM Tris HCl [pH 8.3 at 25°C], 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% [wt/vol] gelatin); 0.2 mM each dATP, dCTP, dGTP, and dTTP; 200 ng of each primer; 2.5 U of *Taq* DNA polymerase (Perkin-Elmer Cetus); and 1 to 2 μg of each DNA.

For the first PCR run (primers env 1 and env 22), the reaction was carried out with 1 μg in a DNA Thermocycler (Perkin-Elmer Cetus) with an initial denaturation step at 94°C and then 35 cycles of 1 min at 94°C, 1 min at 58°C, and 2 min at 72°C (with increased times of 2 s per cycle). Two microliters of the initial PCR product was used for the second PCR run (env 1 and env 2), and the reaction products were analyzed on a 1.5% agarose (ultrapure agarose [Bethesda Research Laboratories, Gaithersburg, Md.]) gel.

Thirteen pairs of oligonucleotide primers, derived either from the HTLV-1 ATK sequence (71) or from the STLV-1 TE4 sequence, were used. The sequences of the primers were as follows: (i) Tax 1 (bp 7630 to 7650), TCCTTCC TCCAGGCCATGCGC, and Rpx 8 (bp 8202 to 8221), AAGAGTACTGTATG AGGCCG; (ii) Px 23 ACDFN (bp 8185 to 8201), TCATTTCTACTCTCACA, and LTR U5E (bp 8735 to 8759), CGCAGTTCAGGAGGCACCACAGGCG; (iii) LTR 417 (bp 422 to 442), CATCCACGCCGGTCGAGTCGC, and GAG 1056 (bp 1038 to 1056), CCGGGGTATCCTTTTGGGA; (iv) TONK 1001 Not 1 (bp 1001 to 1022), ATGTGTCCCATCGACTACTCCC, and 1647 ECORI (bp 1648 to 1668), TTGAGGCGTTCTACGAAGGCG; (v) MT 1610 Not 1 (bp 1610

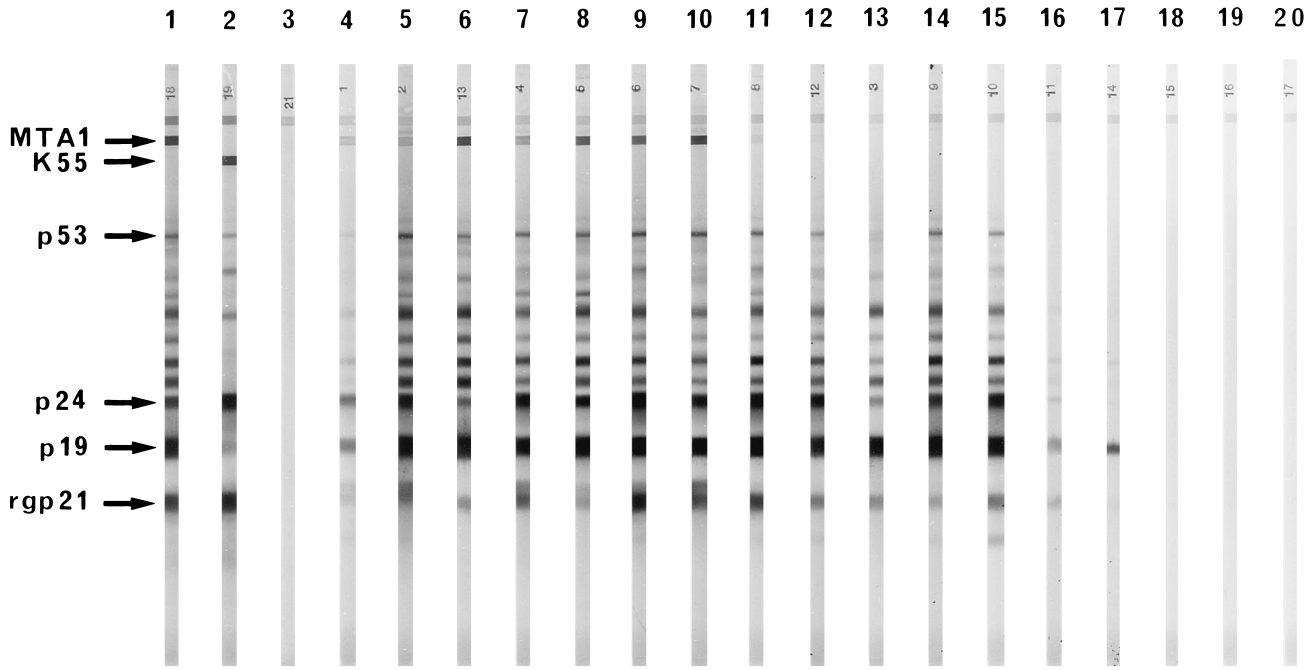

FIG. 1. WB analysis. The WB used was from Diagnostic Biotechnology (HTLV blot 2-3). Lane 1, HTLV-1 positive control; lane 2, HTLV-2 positive control; lane 3, HTLV-1/2 negative control; lanes 4 to 12, nine plasma samples from STLV-1-infected *M. tonkeana* animals with complete HTLV-1 reactivity; lanes 13 to 16, four plasma samples from STLV-1-infected *M. tonkeana* animals without the MTA1 peptide reactivity; lane 17, plasma from animal TG2, exhibiting only p19 reactivity; lanes 18 to 20, three plasma samples from HTLV-1-seronegative animals.

to 1630), TGGGCCTCTATCCTCCAAGGC, and 1981 AS (bp 2006 to 2027), CTGGGGTGGTGGGCTTTAGGCG; (vi) 1983 Not 1 (bp 1983 to 2004), CTC ACTGGAAGCGAGACTGCCC, and 2507 ECORI (bp 2555 to 2577), CTC TAGCCCAAGGACGGCTGGCG; (vii) Pol AG 1 (bp 2474 to 2497), GTCGT GATGCCTTACAACAATGCC, and Pol AG 2 (bp 3577 to 3600), GGGCAT GTAGCCAGACAAGTGGCC; (viii) 3459 TONK (bp 3524 to 3545), CATG CTAACTCTCACTGGCACC, and SK57 (bp 4545 to 4566), TGTCTACCCA TACATGAAGGCG; (ix) Pol AG 3 (bp 4252 to 4275), ATCTACCTGATCCCA TCTCCAGGC, and Pol AG 4 (bp 5190 to 5213), AACTTACCCATGGTGTT GGAGGTC; (x) Agp1 (bp 5106 to 5126), TCTGCCCAGTGGATCCCGTGG, and TONK 2 (bp 6096 to 6117), GGTGGGGACAGGCGACAAAGAG; (xi) ENV 1 (bp 6045 to 6068), TCAAGCTATAGTCTCCTCCCCCTG, and ENV 2 (bp 6591 to 6614), GGGAGGTGTCGTAGCTGACGGAGG; (xii) TONK 1 (bp 6544 to 6565), ACCCTTGTTGCCTTATTCCTCC, and ENV 22 (bp 6774 to 6796), GGCGAGGTGGAGTCCTTGGAGGC; and (xiii) 6755 TONK (bp 6751 to 6772), CCTGCAGCAATCTCCTGCGTTC, and 7655 AS (bp 7659 to 7681) CCATGTATCCATTCCGGAAGGG. All of the upstream (sense) primers were synthesized with the linker sequence TTTGAGCGGCCGC containing the restriction site for *Not*I, and all of the downstream (antisense) primers were synthesized with the linker sequence ACTTAGAATTC carrying the restriction site for *Eco*RI. The PCR was done as described above except that in some instances the annealing temperature was 48°C instead of 58°C to increase the sensitivity of the amplification because of some mismatches between the primers derived from the HTLV-1 ATK sequence and those derived from the STLV-1 TE4 sequence.

(ii) Cloning and sequencing. The purified PCR product was obtained after phenol-chloroform extraction and ethanol precipitation and then digested with 10 U each of *Eco*RI and *Not*I (Boehringer). The digested DNA was purified by centrifugal filtration (Millipore, Bedford, Mass.) and then inserted into a linear *Eco*RI-*Not*I pBluescript vector and molecularly cloned. Positive clones were selected by using ^{32}P-labelled oligonucleotides derived either from the HTLV-1 ATK sequence (71) or from the STLV-1 TE4 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 and to evaluate their accuracies (analysis performed by Fredj Tekaia, Service d'Informatique Scientifique, Institut Pasteur). Multiple alignment of sequences (including the sequence of the new STLV-1 generated in this study and all other available published HTLV-1 [1, 4, 13, 17, 18, 20, 22, 25, 35, 38, 49, 50, 60, 64, 70, 74, 79, 81, 82] and STLV-1 [40, 68, 75, 82, 84] sequences) was performed by using the Clustal V program (29). The resulting aligned sequences were analyzed with different programs of the PHYLIP package (version 3.52c) (Josef Felsenstein, University of Washington [joe@genetics.washington.edu]) and the MEGA (Molecular Evolutionary Genetic Analysis) program (version 1.01) (43).

Two different methods were used to generate phylogenetic trees: the maximum-parsimony method, using the DNAPARS program, and the neighbor-joining (NJ) method (65) (NEIGHBOR program), using the modified NJ approach (58). The latter technique uses the modified Kimura two-parameter distance, which is a maximum-likelihood estimate of the Kimura distance for a

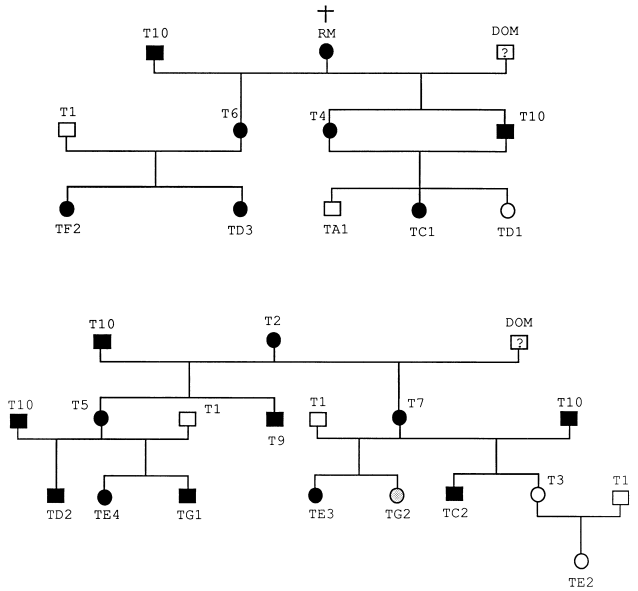

FIG. 2. Genealogical tree (pedigree) with HTLV-I/STLV-I serological results for a troop of 23 *M. tonkeana* animals. Circles, females; squares, males; solid symbols, seropositive; open symbols, seronegative; shaded symbol, indeterminate; ?, not tested; +, dead.

TABLE 2. Cell surface markers, as detected by indirect IF, of three STLV-1-infected cell lines established from *M. tonkeana*

Cell line	% expression of cell surface markers:										
	CD2	CD3	CD4	CD5	CD8	CD11	CD25	CD33	CD56	DR	TCR α/β
TE4	88	0	87	0	0	0	2	0	11	1	0
TD3	91	0	74	0	14	4	6	2	5	1	0
T7CC	77	72	0	74	55	55	51	22	38	75	44

fixed value of alpha/beta (transition/transversion ratio = 2) (58). The SEQBOOT program was used to generate 500 data sets that are randomly resampled versions of the previously aligned sequences. For both the maximum-parsimony and NJ methods, a consensus tree was constructed by using the CONSENSE program with the majority-rule criteria. A distance-based tree was constructed with MEGA by using the NJ method with the Kimura two-parameter model. In order to test the reliability of the final tree topology, the bootstrap technique was also used with 500 replicates.

Nucleotide sequence accession numbers. The GenBank and EMBL accession numbers for the five STLV-1 env 1-env 2 sequences and the complete TE4 STLV-1 sequence are Z46895, Z46896, Z46897, Z46898, Z46899, and Z46900, respectively.

RESULTS

Seroepidemiology. Among the 23 *M. tonkeana* individuals, 17 were found to be HTLV seropositive by the screening assays, with a perfect concordance between the ELISA, the PA, and the IF results (Table 1). Furthermore, when tested by WB, 13 of these 17 animals exhibited a seroreactivity identical to that of the HTLV-1 positive control (Fig. 1), i.e., bands against *gag*-encoded proteins (p19, p24, and p53), *env*-encoded proteins (rgp 21), and, in all cases, the HTLV-1-specific peptide MTA1 and gp46. The remaining 4 of the 17 animals were positive for all bands except MTA1; these samples, however, were considered HTLV-1 seropositive. Only one animal (TG2) had an indeterminate WB pattern, with only a p19 seroreactivity (Fig. 1). The serology was negative for five animals. All 17 seropositive animals had higher titers on the MT2 cell line than on the C19 cell line, confirming that they were infected by an HTLV-1- and not an HTLV-2-related virus (Table 1). They all also had high titers by PA. Animal TG2 with the isolated p19 band on WB had very low titers by PA and IF (Table 1). There was no difference in the WB pattern between the samples collected in April or May 1992 and those collected 1 year later. The five HTLV-1- and STLV-1-seronegative samples were from two males and three females (Table 1; Fig. 2). The genealogical analysis (Fig. 2) of the colony suggested that the virus was transmitted mainly from infected mother to offspring.

Virus isolation. PBMCs from 12 STLV-1-seropositive animals were cultured, and in five cases they were cocultivated with PBMCs from HTLV-1-seronegative blood donors after 2 months of culture alone. Three long-term cultures (named TE4, TD3, and T7CC) were established. The TE4 and TD3 cell lines originated from PBMCs cultured alone, while the T7CC cell line was established from a coculture. The surface phenotype of the T7CC cell line determined by cytofluorography analysis (Table 2) demonstrates that these cells were of the T-cell lineage, with expression of CD2, CD3, CD5, CD8, CD11, and activation markers (CD25 and HLA-DR) without B-cell markers (CD19 and CD20). The TD3 and TE4 cell lines expressed only CD2 and CD4.

The expression of HTLV-1/STLV-1 antigens in the monkey cell cultures varied with the period of culture. HTLV-1-related antigen expression was not detected in short-term cultures (less than 1 month). After 45 days of culture, polyclonal sera from TSP/HAM patients and from STLV-1-infected monkeys and murine monoclonal antibodies against p19 and p24 recognized 1 to 5% of the TD3 and TE4 cells. At 3 months of culture, 15 to 30% of the cells were positive, and at 4 to 5 months, 25 to 40% expressed HTLV-1/STLV-1 antigens. For the T7CC cell line, viral expression appeared later, and at 8 months only 10% of the cells were positive by IF. The level of positive cells was always higher when a polyclonal HTLV-1 or STLV-1 serum was used than when the p19, p24, and gp46 monoclonal antibodies were used (Table 3). Supernatants of both primary (TE4 and TD3) and cocultured (T7CC) cells were positive in an antigen capture assay capable of detecting the major core antigen Gag p24 of both HTLV-1 and HTLV-2 (Fig. 3).

Nucleic acid studies. (i) Southern blot analysis. After digestion with *Eco*RI restriction enzyme at 5 and 8 months of culture, the bands detected in the DNAs of the TE4, TD3, and T7CC cell lines with the 1711 total genomic probe migrated at a position greater than 9 kb (the size of the total HTLV-1 provirus) (data not shown). These results indicate a clonal integration of the STLV-1 provirus in the cell lines.

TABLE 3. Characterization of virus in three STLV-1-infected cell lines established from *M. tonkeana*

Cell line	HTLV-1 Antigen expression[a]			HTLV-1 proviral genome[b]	Protein synthesis (p24)[c]	EM[d]
	TSP/HAM serum	p19	p24			
TE4	+	+	+	+	+	+
TD3	+	+	+	+	+	+
T7CC	+	+	+	+	+	+

[a] Detected by indirect IF with TSP/HAM serum and monoclonal antibodies against p19 and p24.
[b] Detected by PCR with the SK 54 and Pol AG 2 primers.
[c] p24 product in the supernatant fluid was measured by a p24 antigen capture assay (Coulter).
[d] EM, electron microscopy.

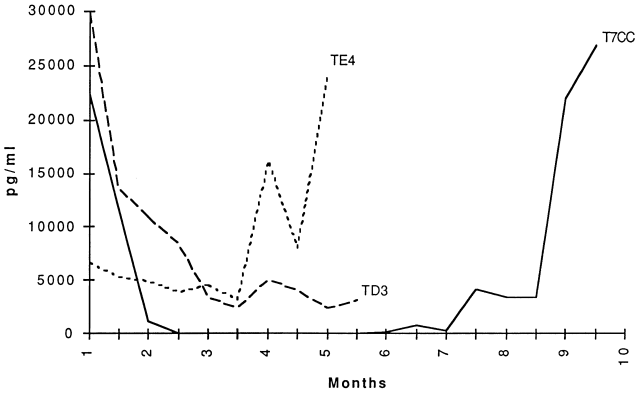

FIG. 3. Time course of detection of p24 viral antigen in the culture supernatants of the three lymphoid T-cell lines (TE4, TD3, and T7CC) established from STLV-1-infected *M. tonkeana* animals.

TABLE 4. Nucleotide similarity in a 522-bp region of the *env* gene between the six STLV-1 isolates from *M. tonkeana* and all available Asian STLV-1 and HTLV-1 isolates

Virus and country of origin	Isolate	% Nucleotide similarity (522-bp *env* region)					
		TE4	T4	T5	T6	TD3	TF2
STLV-1	PTM3	88.7	88.9	88.1	88.5	88.9	88.3
	C194	86.2	86.2	85.4	85.4	86.2	85.8
	MMU39-83	84.7	84.9	83.9	84.3	84.5	84.3
	PCY991	85.4	85.6	84.7	85.1	85.2	85.1
	MMU173	85.2	85.4	84.5	84.9	85.1	84.9
HTLV-1							
Papua New Guinea	Mel 1	87.5	87.7	87.0	87.4	87.7	87.5
	Mel 2	87.7	87.9	87.2	87.5	87.9	87.7
Solomon islands	Mel 3	88.7	88.9	88.1	88.5	88.9	88.3
	Mel 4	88.9	89.1	88.3	88.7	89.1	88.5
	Mel 5	88.7	88.9	88.1	88.5	88.9	88.3
Australia	MHSR1	89.5	89.7	88.9	89.3	89.7	89.5
Japan	ATK	88.7	88.9	88.7	89.1	88.9	88.7
	MT2	88.9	89.1	88.3	88.7	89.1	88.9
	H5	88.9	89.1	88.5	88.9	89.1	88.9
	PHCT	88.9	89.1	88.3	88.7	89.1	88.9

(ii) Sequence analysis of a fragment of the gp21 *env* gene. HTLV-1 provirus-related sequences were detected by PCR in the DNAs extracted from the uncultured PBMCs of the five tested STLV-1-seropositive animals (T5, T6, T4, TF2, and TD3) by using a seminested PCR with the env-1 and env-2 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 these five animals and also for the TE4 cell line. Although the six sequences were very similar to each other, they exhibited 0.2 to 2.5% nucleotidic substitutions when compared with each other, and none of them was identical to any other. The proviral sequences from the animals of the first familial cluster (T6, T4, TF2, and TD3) were not more closely related to each other than to those from the second familial group (T5 and TE4) (Fig. 2). These *env* sequences were compared with all of the available data for HTLV-1 and STLV-1 strains of Asian origin, and no very close similarity with any of the sequences was found. Among the STLV-1 isolates (Table 4), the closest similarity was with PTM3 (88.1 to 88.9%) and to a lesser degree C194 (85.4 to 86.2%), both originating from Indonesia, while the three other STLV-1 isolates (from India and the Sukhumi colony in Russia) were more divergent (83.9 to 85.6% similarity). Interestingly, among the Melanesian HTLV-1 isolates, these new Asian STLV-1 sequences were slightly more related to the Australian MHSR1 strain (88.9 to 89.7%) and to those from the Solomon islands (88.1 to 89.1%) than to those of the Papua New Guinea strains (87 to 87.9%). However, the same level of similarity (88.3 to 89.1%) was found with the Japanese strains (Table 4). After translation of the nucleotide sequences, the amino acids were aligned with the 42 homologous *env* sequences obtained by Koralnik et al. (40). The *M. tonkeana* strains had a threonine (instead of a glycine) at position 328, which is present only in the Melanesian HTLV-1 isolates, the Asian STLV-1 isolates, and HTLV-2. Furthermore, the *M. tonkeana* strains lacked the two specific African mutations, i.e., M at position 330 and R at position 344. These data confirm that these STLV-1 isolates are of Asian origin.

(iii) Overall genetic organization and variability of STLV-1 TE4. We amplified from the cellular DNA of the STLV-1 TE4-infected cell line 13 fragments (a to m) (Fig. 4), encompassing the complete STLV-1 genome, using primers either from the sequence of HTLV-1 ATK or from previously sequenced DNA fragments of the STLV-1 TE4 isolate to increase the sensitivity and specificity of the PCR. A schematic representation of the strategy used to amplify the entire STLV-1 TE4 genome is depicted in Fig. 4, and the primer sequences are described in Materials and Methods.

The sequence of STLV-1 TE4 is the first complete STLV-1 nucleotide sequence (9,025 bases) available, and its overall genetic organization is similar to those of HTLV-1 and HTLV-2, without major deletions or insertions in the LTR, *gag*, *pol*, *env*, and pX regions. The overall divergence at the nucleotide level between STLV-1 TE4 and the HTLV-1 ATK prototype (71) is 11.4%; that from the Mel 5 isolate (the Melanesian prototype from a Solomon Islander [18]) is 10.6%, consistent with more similarity seen in the LTR and in the *pol* gene. The degree of variability in the amino acid sequences of the structural genes ranged from 1.4 to 6.9% compared with those of HTLV-1 ATK and was higher for the regulatory genes (*tax* and *rex*; 7.8 and 14.3%, respectively) and for the other part of the pX region, which in HTLV-1 ATK encodes recently described proteins (8, 42).

(iv) LTR of STLV-1 TE4. The STLV-1 TE4 LTR sequence (748 bases) obtained by sequencing fragments a, b, and c aligned almost perfectly with those of HTLV-1 ATK and HTLV-1 Mel 5 (the most distant HTLV-1 of Melanesian origin [18]) and with the three other available complete LTR sequences from STLV-1 strains, including two from Africa (STLV-1 CH and STLV-1 AG [84]) and one from Indonesia (PTM3 [85]) (Fig. 5). The greatest conservation of the DNA nucleotide sequence, compared with ATK, was in the R region (91.6%), and the lowest was in the U5 region (84.6%) (Table 5). In the U3 region, the three enhancer elements identified in HTLV-1 as the Tax-responsive elements with a conserved central core (except for an A at position 177) were well conserved, as were the c-Ets-responsive elements. A deletion of 9 bases (bp 196 to 204), compared with all of the other available HTLV-1 and STLV-1 LTRs, was observed in STLV-1 TE4. In the R region, containing the Rex-responsive element, a very high degree of similarity was observed in the stem sequence as well as in the Rex binding region between STLV-1 TE4, ATK, Mel 5, and the three other STLV-1 LTR sequences (Fig. 5).

(v) *gag*, *pol*, and *env* genes of STLV-1 TE4. The DNA sequences of the 10 overlapping fragments (c to l) encompassing the 5′ half of the viral genome yielded three open reading

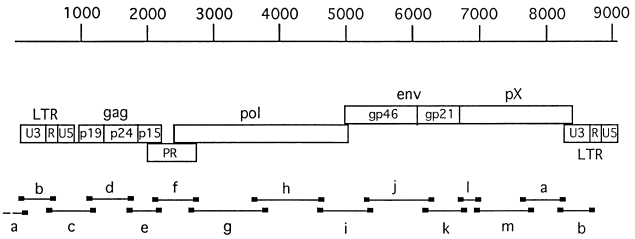

FIG. 4. Strategy for cloning and sequencing of the entire genome of STLV-1 TE4 isolated from *M. tonkeana*, showing the positions of the 13 overlapping DNA fragments (bottom) generated by PCR. The sequences of the oligonucleotide primers used are given in Materials and Methods. Numbers at the top are base pairs.

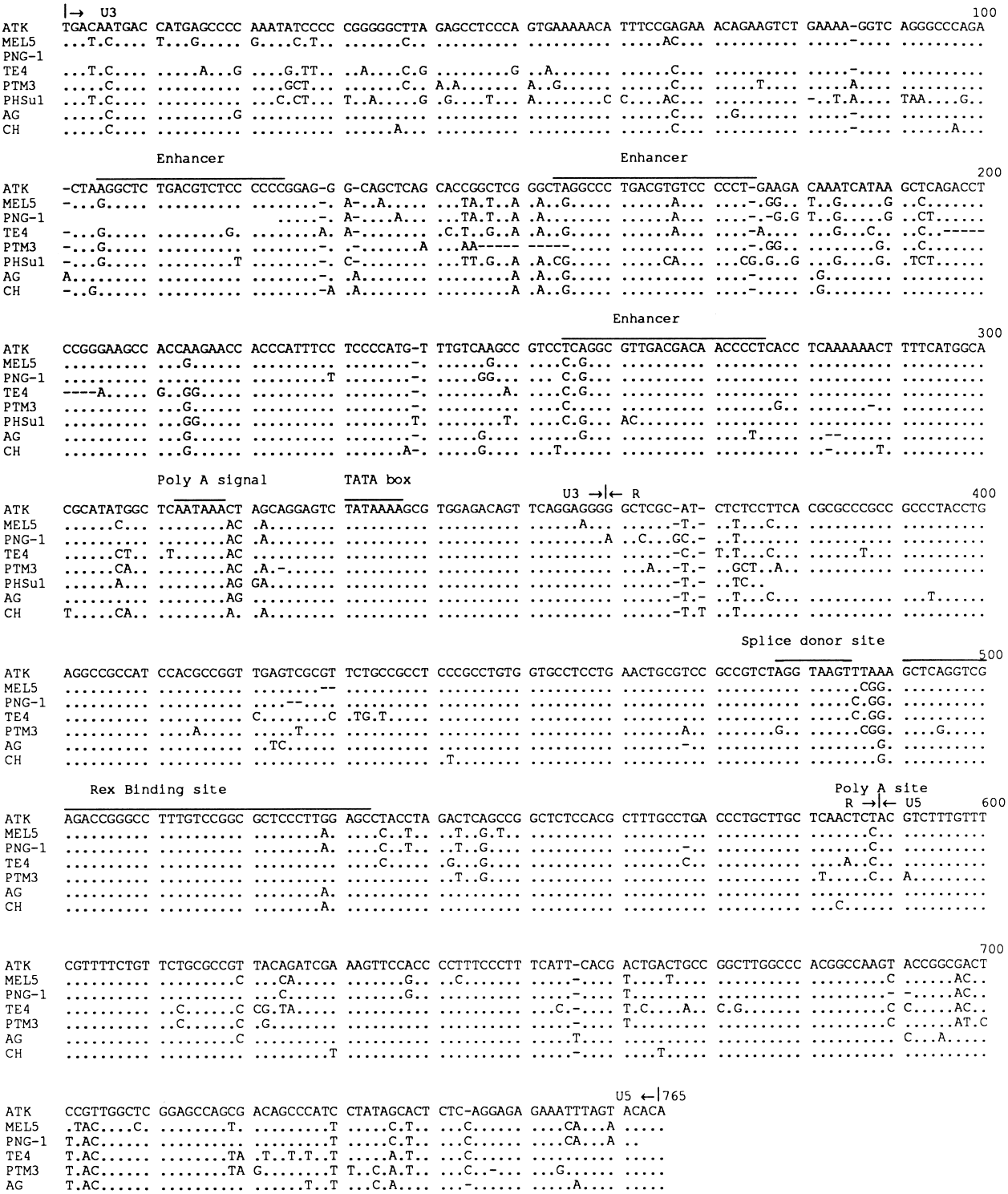

FIG. 5. Nucleotide alignment of the LTRs of STLV-1 TE4, HTLV-1 ATK, HTLV-1 Mel 5, HTLV-1 PNG1, STLV-1 AG, STLV-1 CH, STLV-1 PHsu1, and STLV-1 PTM3. Dots represent nucleotides identical to those of the ATK sequence. The bar represents a deletion.

frames (ORFs) corresponding to the *gag*, protease, and *pol* genes. The *gag* precursor consisted of 1,289 nucleotides coding for 429 amino acids. The protease gene (905 bases) coded for 302 amino acids. The *pol* precursor was composed of 2,687 nucleotides encoding 896 amino acids. The *env* gene (1,464 bases) encoded 487 amino acids, including gp46 and gp21. A deletion of 3 bases was present in STLV-1 TE4 at position 5950 (HTLV-1 ATK numbering).

The STLV-1 TE4 structural genes displayed a nucleotide divergence ranging between 7.8 and 13.1% (Table 6) and an

TABLE 5. Nucleotide similarity between the LTR of STLV-1 TE4 and those of HTLV-1 ATK and Mel 5 and STLV-1 PTM3, CH, and AG

Isolate	% Nucleotide similarity to STLV-1 TE4 in the following LTR region:			
	Total	U3	R	U5
HTLV-1 ATK	88.6	88.7	91.6	84.6
HTLV-1 Mel 5	90.3	90.4	93.3	86.3
STLV-1 PTM3	88.4	88.3	88.1	89.1
STLV-1 CH	88.6	88.4	91.6	85.1
STLV-1 AG	89.8	90.1	91.1	87.4

amino acid divergence ranging between 1.4 and 9.2% (Table 7) with respect to the equivalent genes of HTLV-1 ATK, Mel 5, and PTM3. The most divergent among the structural genes was the p19 *gag* gene, but its carboxy terminus, corresponding to a B-cell immunodominant epitope in HTLV-1 ATK (45) (residues 102 to 117, PPSSPTHDPPDSDPQI), was highly conserved, with only two amino acid changes (S to P at position 106 and D to S at position 110). For the *pol* gene, the immunodominant B-cell epitope in HTLV-1 ATK (residues 487 to 502, KQILQRSFPLRPPHK) exhibited three amino acid changes compared with the ATK sequence, at positions 488, 493, and 498. For the *env* gene, the two B-cell immunodominant epitopes SP4a and VIEA (61, 70) were highly conserved in STLV-1 TE4 compared with HTLV-1 ATK, Mel 5, and PTM3, while the VIE10 epitope was less conserved, with few amino acid changes. Thus, the amino acid sequence of the immunodominant epitope present in the synthetic peptide MTA1 (residues 169 to 209), used in a WB for diagnostic assay (6), demonstrated total identity in STLV-1 TE4 compared with the ATK prototype. Furthermore, the type-specific neutralizing epitope (residues 88 to 98) was identical in STLV-1 TE4 and PTM3 and HTLV-1 Mel 1 and Mel 5, with, however, a threonine replacing an isoleucine at position 89 in HTLV-1 ATK.

(vi) Regulatory genes and the other novel genes of STLV-1. The 3′ end of the HTLV-1 genome (pX) carries genetic information for at least seven proteins, including the two regulatory proteins $p40^{tax}$ and $p27^{rex}$, the $p21^{rex}$, and four recently described proteins, $p13^{II}$, $p30^{II}$, $p12^{I}$, and Rex^{orfI} (8, 14, 41, 42).

The amino acid alignments for $p40^{tax}$, $p27^{rex}$, and $p21^{rex}$ of STLV-1 TE4 and PTM3 and HTLV-1 Mel 5 and ATK are presented in Fig. 6, and their percentages of nucleotide and amino acid homology are presented in Table 8. These data demonstrate that the greatest degree of similarity between these different PTLV was observed in the first 90 amino acids of the Tax protein, which contains some of the active sites for functional activity, including the zinc finger region. The carboxy terminus region (residues 321 to 352) exhibited a higher degree of amino acid diversity between the different available HTLV-1 and STLV-1 strains.

The first exon (residues 1 to 20) of the $p27^{rex}$ protein of STLV-1 TE4, considered the nucleolar targeting sequence, exhibited only two amino acid changes compared with ATK (Fig. 6), while the carboxy terminus of the Rex protein was less conserved.

Concerning the potential proteins encoded by pX ORF I and ORF II in the HTLV-1 genome, the ATG initiation codon of the $p12^{I}$ protein was replaced in STLV-1 TE4 by a proline because of a mutation in the nucleotide sequence (T to C at position 6858 of ATK). Interestingly, the same mutation was also present in the sequence of STLV-1 PTM3 (85). Furthermore, for $p13^{II}$ the ATG initiation codon present in ATK at position 7313 was replaced by an isoleucine (due to a mutation of G to A) in the STLV-1 TE4 strain. The highest degree of amino acid similarity was observed for the region (ORF II) which in HTLV-1 encodes both $p13^{II}$ and $p30^{II}$, while the region (ORF I) encoding $p12^{I}$ and Rex^{orfI} in HTLV-1 ATK was more divergent.

(vii) Phylogenetic relationships between STLV-1 TE4 and the other HTLV-1 and STLV-1 isolates. Three portions of the STLV-1 TE4 genome were used for phylogenetic analysis: a fragment of 315 bp (nucleotides 120 to 434) of the LTR encompassing most of the U3 and the R region (81), a fragment of 522 bp (nucleotides 6046 to 6567) encompassing most of gp21 and the carboxy terminus (20, 40) of gp46, and a smaller fragment of 229 bp (nucleotides 5247 to 5475) within the gp46 (75). Since tree-building algorithms rely on different assumptions, we used two different methods (NJ and maximum parsimony) to increase the reliability of the derived tree topologies. In the LTR analysis, the two phylogenetic methods suggested evidence of five genomic clades with, however, weak consensus values. As seen in Fig. 7A, the first group includes the Cosmopolitan HTLV-1 strains, comprising all of the American and Caribbean samples except one from Jamaica; the second group includes the Japanese strains (the bootstrap values were low for these two groups); the third group comprises the West African strains, including the isolates from the Ivory Coast and Mali and the Jamaican isolate HS 35 (originating from an individual of African ancestry (50) (bootstrap value, 77); the fourth clade includes the Central African strains, comprising the Zairian and Gabonese strains as well as the three STLV-1 isolates (AG, CH, and TAN 90, originating from Gabon and the Central African Republic) (bootstrap value, 74); and the fifth clade includes all of the Asian strains, with the two distant HTLV-1 strains from Papua New Guinea and the Solomon island Melanesian HTLV-1 (bootstrap value, 94), the presently [17]described STLV-1 TE4 from Sulawesi, and STLV-1 PTM3 from Indonesia. The topologies of the two trees obtained with the different methods were very similar; however, the only exception to the concordance between geographical origin and molecular clustering was the position of TAN 90

TABLE 6. Nucleotide sequence comparison of the STLV-1 TE4 structural genes and those of HTLV-1 ATK, HTLV-1 Mel 5, and STLV-1 PTM3

Isolate	% Nucleotide similarity to STLV-1 TE4:								
	gag				Protease gene	*pol*	*env*		
	Precursor	p19	p24	p15			Precursor	gp46	gp21
HTLV-1 ATK	90.0	87.1	90.8	92.2	90.4	88.8	89.3	89.5	88.9
HTLV-1 Mel 5	90.2	87.9	90.8	92.2	90.3	90.1	88.9	88.5	89.5
STLV-1 PTM3							87.7	86.9	89.3

TABLE 7. Amino acid sequence comparison of the STLV-1 TE4 structural gene products and those of HTLV-1 ATK, HTLV-1 Mel 5, and STLV-1 PTM3

Isolate	% Amino acid similarity to STLV-1 TE4:								
	Gag				Protease	Pol	Env		
	Precursor	p19	p24	p15			Precursor	gp46	gp21
HTLV-1 ATK	96.5	93.1	98.6	96.5	90.1	94.9	95.5	95.5	95.5
HTLV-1 Mel 5	95.1	90.8	97.7	95.3	90.7	95.2	96.1	95.2	97.7
STLV-1 PTM3							96.3	95.2	98.3

(68), which in the NJ analysis (data not shown) was not within the African cluster of HTLV-1 and STLV-1. Such a positioning problem for TAN 90 has been previously reported by Song et al. (75). The comparative analysis of the gp21 *env* gene, performed with the 90 available HTLV-1 and STLV-1 sequences, including 58 HTLV-1 and 32 STLV-1 sequences, plus the 6 new sequences from the *M. tonkeana* isolates showed the presence of several phylogenetic clades, some of them with very high bootstrap values. However, as seen in Fig. 7B and C, three main groups can be identified. The first group comprised all of the Cosmopolitan and the Japanese HTLV-1 isolates (clade 1 in a recent published analysis [40] corresponds to part of this group). The second group included the African HTLV-1 isolates (a few of these, mostly from West Africa, belonged to the Cosmopolitan type) and all of the African STLV-1 strains. This cluster included HTLV-1 clade 2 and STLV-1 groups S2 to S7 of a recently published analysis (40). The third group comprised the most distant STLV-1 and HTLV-1 isolates, i.e., the Melanesian (Papua New Guinea, Solomon Islands, and Australia) HTLV-1 isolates (bootstrap value, 99 to 100) and all of the Asian STLV-1 isolates. Within this group, our six STLV-1 isolates from *M. tonkeana* always clustered together (bootstrap value, 100) at a position located between the Melanesian HTLV-1 group and all of the other more distant Asian STLV-1 isolates (clade 3 and the S1 group, respectively, in reference 40). Again, the tree topologies were very similar by the two methods of analysis (Fig. 7B and C), indicating that the new STLV-1 isolates from *M. tonkeana* formed a monophyletic group (bootstrap value, 100) in the Asian HTLV-1 and STLV-1 strains related to the Melanesian HTLV-1 strains. The phylogenetic analysis of a smaller part of gp46 (229 bp) included Japanese STLV-1 isolates from *Macaca fuscata*, a spe-

TAX

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ATK    MAHFPGFGQS LLFGYPVYVF GDCVQGDWCP ISGGLCSARL HRHALLATCP
MEL5   .......... ..Y....... .......... .......... ..........
TE4    .......... ..Y....... .......... .......... ..........
PTM3   .......... ..Y....... .N........ .......... ..........

ATK    EHQITWDPID GRVIGSALQF LIPRLPSFPT QRTSKTLKVL TPPITHTTPN
MEL5   .....-.... .......... .......... .......... ...T......
TE4    .......... ......T... .......... .......... ...T...A..
PTM3   ........T. E......... .......L.. .......... ...A......

ATK    PPSFLQAMRK YSPFRNGYME PTLGQHLPTL SFPDPGLRPQ NLYTLWGGSV
MEL5   ....F..V.Q H......C.. .....Q..S. .......... .......S..
TE4    ....F..I.. .......... ...R...... .......... ......E...
PTM3   ....F..V.. .......... .....Q.... .......... .......N..

ATK    VCMYLYQLSP PITWPLLPHV IFCHPGQLGA FLTNVPYKRI EELLYKISLT
MEL5   .......... ........Q. .......... .........M .......F.N
TE4    .......... .......... .......... .........M .......F.N
PTM3   .......... .......... .......... .........M .......F.N

ATK    TGALIILPED CLPTTLFQPA RAPVTLTAWQ NGLLPFHSTL TTPGLIWTFT
MEL5   .........G .........I ...A...... ......Q... .........S
TE4    .....V.... .........T ...A.M.... ......Q... .......A..
PTM3   ...T...... .......R.T ...AS...R. ......Q... ..........

ATK    DGTPMISGPC PKDGQPSLVL QSSSFIFHKF QTKAYHPSFL LSHGLIQYSS
MEL5   .......... .......... .......... .........V ..........
TE4    .......... .......... .......N.. .......... ..........
PTM3   .....V.... .R........ .......... .......... ..........

ATK    FHSLHLLFEE YTNIPISLLF NEKEADDNDH EPQISPGGLE PPSEKHFRET
MEL5   ......P... .......... .KR....T.Y G.R.P..... .......H..
TE4    ..N....... .....V.... .....N.T.. ..EMF.E... ..GK......
PTM3   ..N....... .......... .KE..N.T.. ...ML....K ..NG......

ATK    EV
MEL5   ..
TE4    ..
PTM3   D.
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p27rex

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ATK    MPKTRRRPRR SQRKRPPTPW PTSQGLDRVF FSDTQSTCLE TVYKATGAPS
MEL5   ......G... .......... .......K.. .T.I...... ..........
TE4    ......G... ......S... .......K.. .T........ ....V.....
PTM3   ......G... .......... ....V...A. .M.....Y.. ..C.......

ATK    LGDYVRPAYI VTPYWPPVQS IRSPGTPSMD ALSAQLYSSL SLDSPPSPPR
MEL5   .......... .......... ....R..... .......... ..G.......
TE4    P....Q.... .......A.. .......... .......... ..........
PTM3   ....A...CT .......... .......P.N ......C... .........K

ATK    EPLRPSRSLP RQSLIQPPTF HPPSSRPCAN TPPSEMDTWN PPLGSTSQPC
MEL5   ...K...... HRP....... .......Y.. ......GA.S .....S..A.
TE4    ......K... .RP....... R......Y.S I...G....S ..C..N....
PTM3   .......... .RL....... .......YE. .....T...S ....NN....

ATK    LFQTPDSGPK TCTPSGEAPL SACTSTSFPP PSPGPSCPT
MEL5   PSP..A.... .........S .....I.... ........R
TE4    P.P..A.... ......K.LS .....IN... .........
PTM3   P.P..A.... ..I....T.. ......N... ........M
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p21rex

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ATK    MDALSAQLYS SLSLDSPPSP PREPLRPSRS LPRQSLIQPP TFHPPSSRPC
MEL5   .......... ....G..... .....K.... ..HRP..... .........Y
TE4    .......... .......... ........K. ...RP..... ..R......Y
PTM3   .N......C. .......... .K........ ...RL..... .........Y

ATK    ANTPPSEMDT WNPPLGSTSQ PCLFQTPDSG PKTCTPSGEA PLSACTSTSF
MEL5   ........GA .S.....S.. A.PSP..A.. .......... .S.....I..
TE4    .SI...G... .S..C..N.. ..P.P..A.. ........K. LS.....IN.
PTM3   E......T.. .S....NN.. ..P.P..A.. ....I....T.........N.

ATK    PPPSPGPSCP T
MEL5   .......... R
TE4    .......... .
PTM3   .......... M
```

FIG. 6. Amino acid alignments for p40^{tax}, p27^{rex}, and p21^{rex} of STLV-1 TE4, HTLV-1 ATK, HTLV-1 Mel 5, and STLV-1 PTM3. The sequences are shown in the single-letter code. Dots represent identical amino acids.

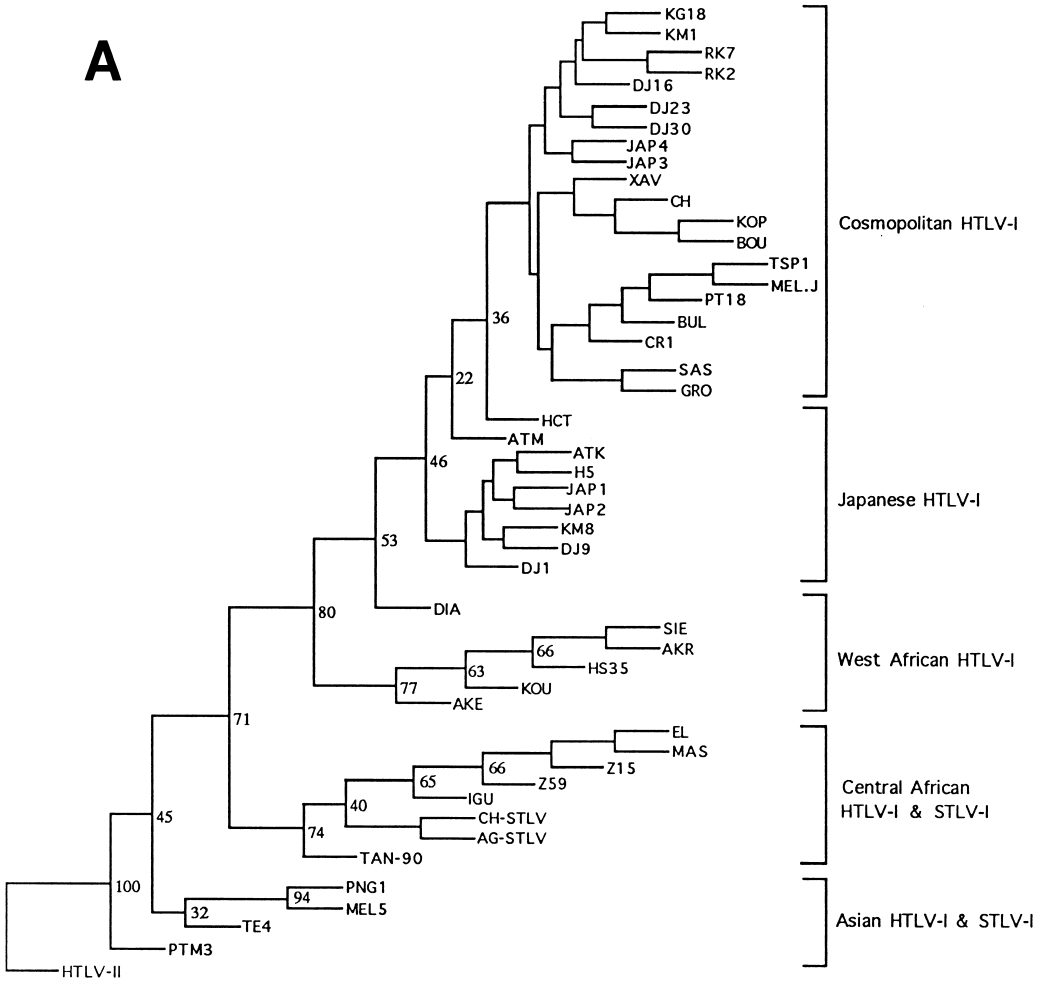

FIG. 7. Phylogenetic trees. (A) Phylogenetic tree constructed by the maximum-parsimony method with a fragment of 315 bp of the LTR encompassing most of the U3 region and the R region (124 varied sites) (81). (B) Phylogenetic tree constructed by the modified NJ method (58) with a fragment of 522 bp encompassing most of gp21 and the carboxy terminus of gp46 (20, 40). (C) Phylogenetic tree constructed with the MEGA program (43) with a fragment of 522 bp encompassing most of gp21 and the carboxy terminus of gp46 (20, 40). All of the available published STLV-1 and HTLV-1 sequences for each genomic portion analyzed were used for these studies, and the HTLV-2 MO isolate (73) was used as an outgroup to root the trees. The numbers indicated at some nodes represent their frequencies of occurrence out of 500 trees.

cies not studied in the two previous (LTR and gp21) analyses, and demonstrated that STLV-1 TE4 was phylogenetically distant from the Japanese STLV-1, [17]which formed the earliest radiation in this analysis. Furthermore, by the NJ method, STLV-1 TE4 was located at a position indicating a divergence more recent than the Melanesian radiation.

TABLE 8. Nucleotide and amino acid sequence comparison of the STLV-1 TE4 Tax, p27^{rex}, and p21^{rex} regulatory genes and those of HTLV-1 ATK, HTLV-1 Mel 5, and STLV-1 PTM3

Isolate	% Similarity to STLV-1 TE4:					
	Tax		p27^{rex}		p21^{rex}	
	Nucleotide	Amino acid	Nucleotide	Amino acid	Nucleotide	Amino acid
HTLV-1 ATK	91.6	92.0	93.6	85.7	92.8	82.9
HTLV-1 Mel 5	90.6	89.8	93.7	86.8	92.2	83.8
STLV-1 PTM3	88.7	90.9	91.5	82.0	91.3	82.0

DISCUSSION

This paper reports the isolation and the complete sequence of a new STLV-1 present in a troop of *M. tonkeana*. The presence of the virus in 74% of the animals of the troop (including both of the two tested founders) gives evidence, as stressed by the genealogical analysis over three generations, of a dominant mother-to-offspring transmission. This strongly suggests that this virus was present in the *M. tonkeana* troop since their arrival in the Primatology Center in Strasbourg, France, in the early 1970s and was not acquired in captivity. Genetic analysis revealed that this virus is identical in the different STLV-1-seropositive animals and represents a distinct new STLV-1 of Asian origin. The first complete nucleotide sequence of an STLV-1 (9,025 bp), obtained for the TE4 isolate, indicated an overall genetic organization similar to those of HTLV-1 and HTLV-2, with, however, a nucleotide variability ranging from 7.8 to 13.1% compared with those of the HTLV-1 ATK and STLV-1 PTM3 Asian prototypes, depending on the structural genes studied. Among the structural and regulatory genes of PTLV (including the STLV-1 from *M.*

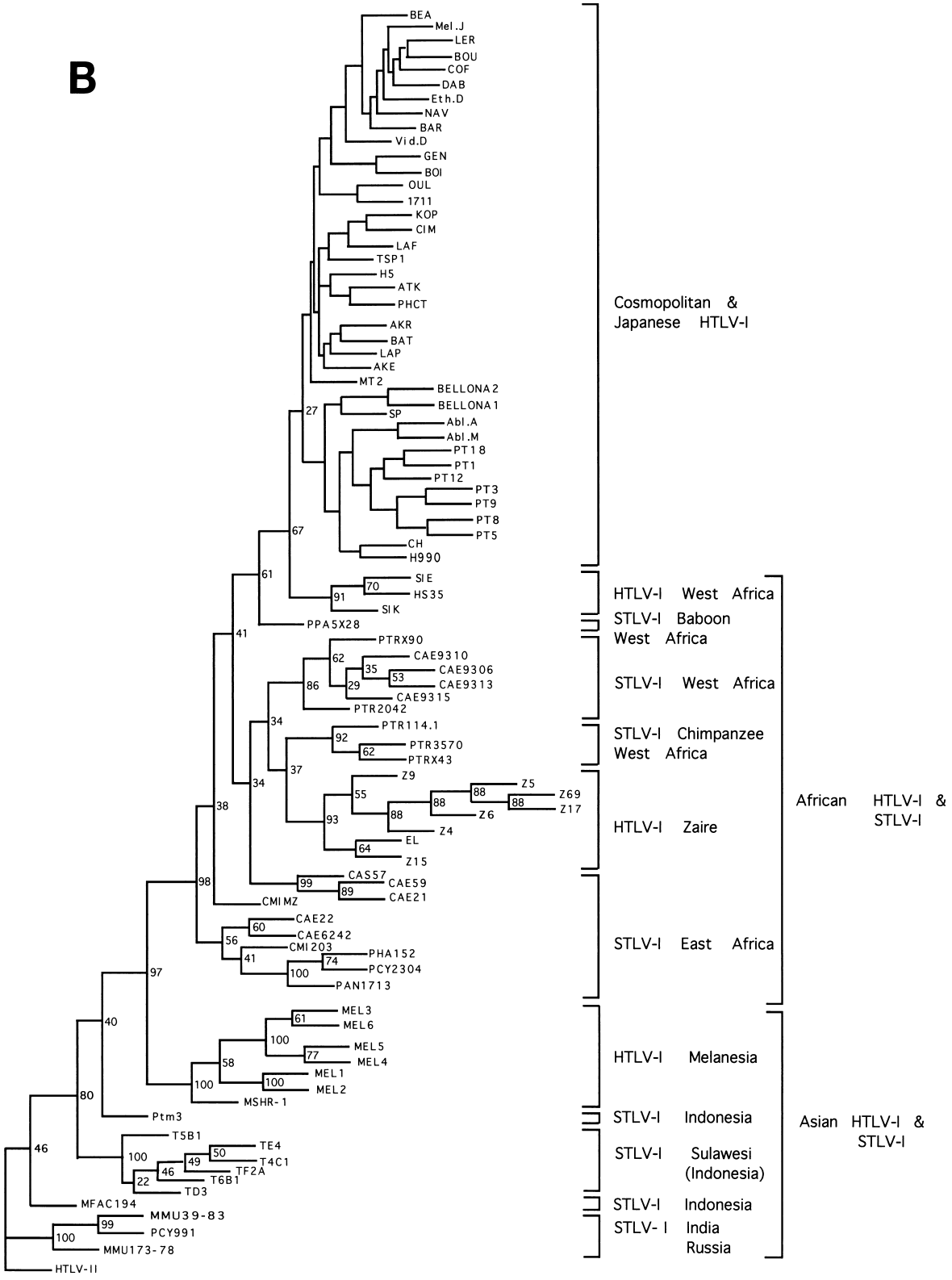

FIG. 7—*Continued.*

tonkeana), the Tax protein (encoded by ORF IV of the pX region), the immunodominant B- and T-cell epitopes, and the neutralizing domain present in the *env* gene were fully conserved (45, 61). This very high degree of amino acid homology of the Tax proteins of all the HTLV-1 and STLV-1 strains, especially in the first 90 residues, which are known to contain some of the critical parts of Tax activity, reflects a very high genetic pressure common to the different PTLV (63, 67). In

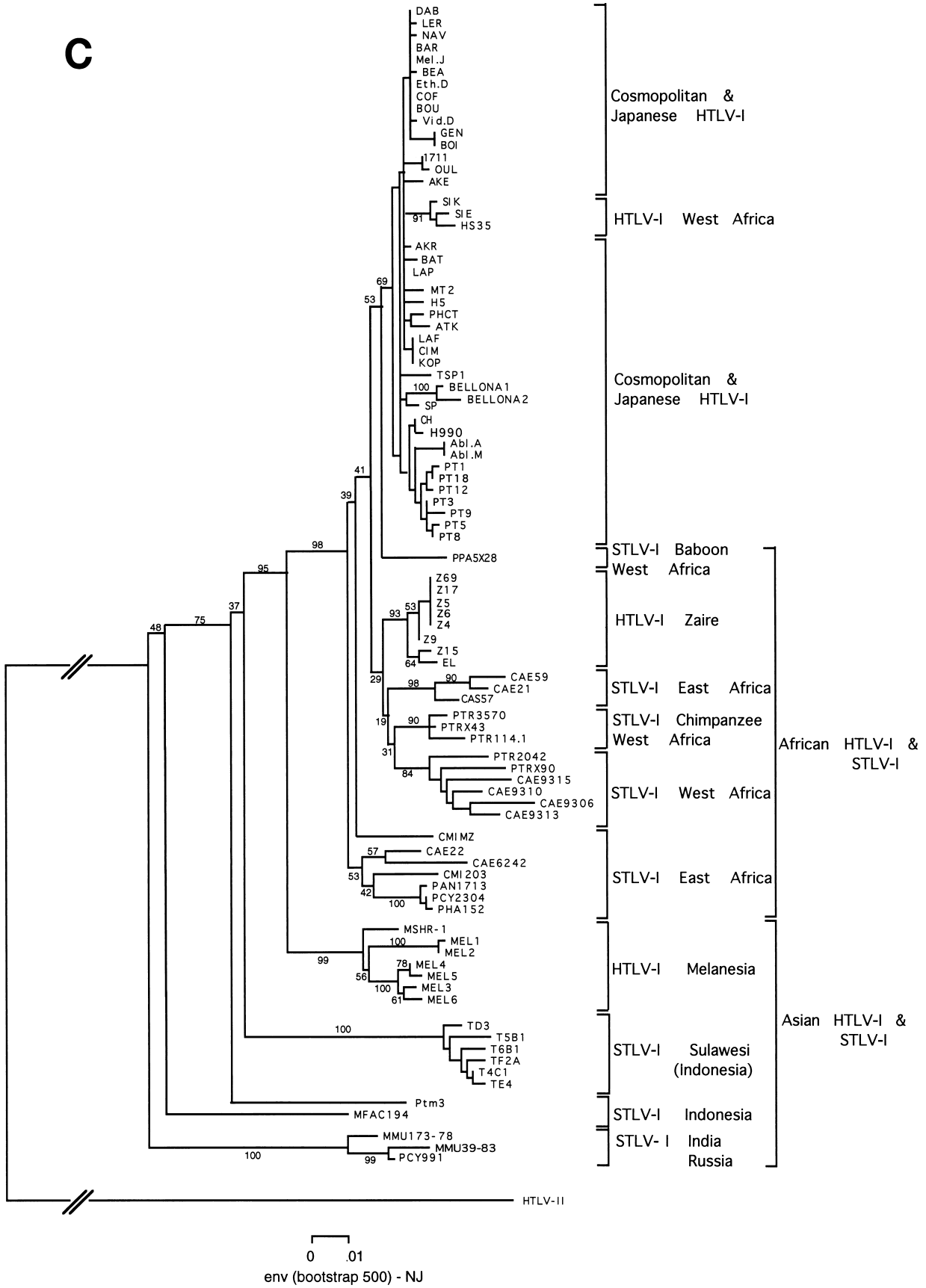

FIG. 7—*Continued.*

contrast, the pX region, containing ORF I and ORF II in HTLV-1 ATK, was more divergent. This region of approximately 650 nucleotides, formerly called the noncoding or nonconserved region, located between the 3′ end of the *env* gene and the second exon of the *tax*/*rex* genes has recently been shown to encode four proteins ($p12^{I}$, Rex^{orfI}, $p13^{II}$, and $p30^{II}$) in HTLV-1 ATK (8, 42). The significance of the lesser conservation of this region, which diverges in a pattern consistent with its overall nucleotidic evolutionary relationship, remains unknown. Furthermore, the functional impact of the potential absence of $p12^{I}$ and $p13^{II}$ (due to the lack of ATG for both of these proteins) on the STLV-1 TE4 viral cycle and replication is unknown. However, in the absence of a reverse transcription PCR analysis, we cannot conclude that this virus is devoid of a gene functionally equivalent to the $p12^{I}$ gene of HTLV-1. It is worth noting that the ATG for $p12^{I}$ is also lacking in STLV-1 PTM3 (85) and HTLV-1 Mel 5 (18), both of which are of Asian origin. The small, hydrophobic $p12^{I}$ protein is localized in the cellular endomembrane (41), and recent data indicate that it has some functional analogy with the E5 oncoprotein of bovine papillomavirus, binds to the 16-kDa component of the cellular vacuolar H^{+} ATPase (14), and interacts specifically with the interleukin-2 R beta and gamma, but not alpha, chains (13a).

Monkey TE4 and three other animals of the *M. tonkeana* troop (Table 2) exhibited no detectable antibody to the MTA1 peptide on the WB, as is the case for some humans infected by HTLV-1 (6). We thought that such a lack of MTA1 reactivity might indicate an antigenic variation in the critical amino acids required for antibody binding of variant forms of STLV-1 having significant envelope divergence. However, the comparison of the amino acid sequences corresponding to the MTA1 peptide (positions 162 to 209) revealed a total identity between STLV-1 TE4 and HTLV-1 ATK, demonstrating that in STLV-1 TE4, the lack of immune seroreactivity to the specific epitope present in the MTA1 peptide is not due to sequence variation within this epitope. Thus, in this animal, antibody titers to the gp46 epitope (MTA1) might be extremely low, or alterations in other portions of the envelope proteins might modify the accessibility of the MTA1 epitope to the animal's immune system. Alternatively, a genetic restriction of the immune system to this specific epitope still remains a possibility. Similar findings have been recently described for two asymptomatic Seminole Amerindians with HTLV-2 infection (44), suggesting that in primates some of the Env immunodominant epitopes may be not universally recognized. It is critical on a diagnostic level and in the public health area to keep in mind such possible lack of seroreactivity, despite a totally conserved epitope, when interpreting the HTLV-1 WB patterns of human sera in blood bank and seroepidemiological surveys.

The origin and worldwide dissemination of the PTLV is still a matter of controversy (18, 19, 23, 47, 63, 67, 72, 82). However, on the basis of the growing number of phylogenetic analyses, most authors suggest an Asian origin of these viruses followed by a migration to Africa (18, 72, 89). The close relationship of the different Melanesian HTLV-1 strains to one another suggests a common ancestor. Such an ancestor may have originated from an STLV-1 following an interspecies transmission between human and nonhuman primates somewhere in Asia. Since there have never been any nonhuman primates in Melanesia, such an event must have occurred during the migration of the ancestors of the present-day Melanesians, before their original arrival in Melanesia or Australia at least 10,000 to 30,000 years ago (18, 72, 89). Despite our finding of a close phylogenetic relationship between the new STLV-1 from *M. tonkeana* and the Melanesian HTLV-1 strains, these two groups of viruses exhibited around 10% nucleotide divergence, and to date no STLV-1 as closely related to the HTLV-1 Melanesian strains as some STLV-1 chimpanzee strains are to some Zairian HTLV-1 strains (40) has been found in Asia. This might be due in part to the long period of independent viral evolution in remote Melanesian populations which took place after the occurrence of the interspecies transmission in Asia, while in Africa some of these interspecies transmissions might have occurred more recently (40, 67, 75).

Studies of STLV-1 strains of Indonesian origin from other species of Celebes macaques are ongoing in order to clarify the origin and evolution of the primate T-lymphotropic retroviruses in this region. Furthermore, such studies may provide animal models for HTLV-1 vaccine development (56, 57) and for a better understanding of the physiopathology of the STLV-1- and HTLV-1-associated pathologies.

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