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Genetic Analysis and Molecular Phylogeny of Simian T-Cell Lymphotropic Virus Type I: Evidence for Independent Virus Evolution in Asia and Africa[1]

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Type C retroviruses, designated simian T-cell lymphotropic virus type I (STLV-I), have been isolated from several genera of Old World monkeys and apes, but not from New World monkeys and prosimians. To determine the genomic diversity and molecular evolution of STLV-I and to clarify their genetic relationship to human T-cell lymphotropic virus type I (HTLV-I), we enzymatically amplified, then directly sequenced selected regions of the *gag, pol, env,* and *pX* genes of STLV-I strains from Asia and Africa. STLV-I strains Si-2, Matsu, and JM86 from Japanese macaques, which exhibited sequence similarities ranging from 98.5 to 99.8% among themselves, diverged by 12.9 to 13.3% from STLV-I strain MM39-83 from a naturally infected rhesus macaque, by 9.7 to 11.2% from STLV-I strains from Africa, and by 8.8 to 11.2% from HTLV-I strains originating in Japan, India, Africa, the Caribbean, the Americas, Polynesia, and Melanesia. By contrast, the interspecies nucleotide sequence similarity among African STLV-I strains from green monkey, yellow baboon, sooty mangabey, and common chimpanzee was remarkably high, ranging from 96.9 to 97.4%, and these STLV-I strains diverged by only 2.2 to 2.8% from HTLV-I strain EL from equatorial Zaire. Phylogenetic trees constructed by using the neighbor-joining and maximum parsimony methods indicated that the Asian STLV-I strains diverged from the common ancestral virus prior to African STLV-I and cosmopolitan and Melanesian HTLV-I strains. Thus, our data are consistent with an archaic presence of STLV-I in Asia, probably predating macaque speciation, with subsequent independent virus evolution in Asia and Africa.

INTRODUCTION

Previous seroepidemiological surveys, conducted among free-ranging or captive Asian and African nonhuman primates, have indicated the existence of viruses antigenically related to human T-cell lymphotropic virus type I (HTLV-I; Hayami *et al.*, 1984; Ishida *et al.*, 1985, 1986; Ishikawa *et al.*, 1987; Miyoshi *et al.*, 1983a; Yamamoto *et al.*, 1988), the etiological agent of adult T-cell leukemia/lymphoma and tropical spastic paraparesis/HTLV-I-associated myelopathy. Type C retroviruses, designated simian T-cell lymphotropic virus type I (STLV-I), have since been isolated from several genera of catarrhines (Old World monkeys and apes), including Japanese macaque, rhesus macaque, Formosan rock macaque, crab-eating or cynomolgus macaque, bonnet macaque, stump-tailed macaque, pig-tailed macaque, moor macaque, yellow baboon, green monkey, tantalus monkey, Sykes' monkey, siamang, and chimpanzee (Daniel *et al.*, 1988; Guo *et al.*, 1984; Ishikawa *et al.*, 1987; Miyoshi *et al.*, 1983b; Saksena *et al.*, 1993; Tsujimoto *et al.*, 1985), but not from platyrrhines (New World monkeys) or prosimians. Although STLV-I has been linked to malignant lymphoma and leukemia in several macaque species, baboon, green monkey, and gorilla (Homma *et al.*, 1984; Lee *et al.*, 1985; Sakakibara *et al.*, 1986; Schätzl *et al.*, 1992), a chronic spastic myeloneuropathy resembling the disease in humans has not been reported in STLV-I-infected nonhuman primates. To what extent host genetic or viral genetic factors contribute to this differential disease potential is unclear.

Analyses of LTR and *env* gene sequences of STLV-I isolates from African and Asian nonhuman primates have revealed that STLV-I strains from a chimpanzee and an African green monkey were more closely related to cosmopolitan strains of HTLV-I than to an STLV-I strain from an Indonesian pig-tailed macaque (Watanabe *et al.*, 1985, 1986). The recent discovery of highly divergent molecular variants of HTLV-I from isolated populations in Papua New Guinea and the Solomon Islands (Gessain *et al.*, 1991; Nerurkar *et al.*, 1993b; Saksena *et al.*, 1992; Sherman *et al.*, 1992) has prompted a renewed inquiry into the evolution of these

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TABLE 1

COUNTRY OR REGION OF ORIGIN AND HOST SPECIES OF STLV-I AND HTLV-I STRAINS USED IN GENETIC AND PHYLOGENETIC ANALYSIS

Virus	Strain	Country or region of origin	Host species
STLV-I	Si-2	Japan	*Macaca fuscata*
	JM86		
	Matsu		
	MM39-83	Southern Asia	*Macaca mulatta*
	PtM3	Indonesia	*Macaca nemestrina*
	AGM22	Kenya	*Cercopithecus aethiops*
	Tan90	Central Africa	
	Somayer	West Africa	*Cercocebus atys*
	KIA	Southern Africa	*Papio cynocephalus*
	ChM114-1	Sierra Leone	*Pan troglodytes*
HTLV-I	ATK	Japan	*Homo sapiens*
	MT-2		
	CMCH 13	India	
	CR	United States	
	CH	Caribbean	
	HS-35		
	ST	Chile	
	EL	Zaire	
	BEL 1	Bellona Island	
	MEL 1	Papua New Guinea	
	MEL 5	Solomon Islands	

type C retroviruses, occasionally referred to as primate T-cell lymphotropic viruses (Guo *et al.*, 1984). To determine the genomic diversity of STLV-I and to clarify their phylogenetic relationship to cosmopolitan and Melanesian strains of HTLV-I, we enzymatically amplified by polymerase chain reaction (PCR), then directly sequenced representative regions of the *gag, pol, env,* and *pX* genes of STLV-I strains from Asia and Africa. Our sequence and phylogenetic analyses are consistent with an archaic presence of STLV-I in Asia, probably predating the time of macaque speciation, with subsequent independent evolution of STLV-I in Asia and Africa.

MATERIALS AND METHODS

Virus strains

Six STLV-I strains from Asian and African nonhuman primates were studied (Table 1). Strains Si-2 (Miyoshi *et al.*, 1983b) and JM86 (Tsujimoto *et al.*, 1985) were isolated from captive Japanese macaques (*Macaca fuscata*) housed at the Primate Research Institute of Kyoto University; strain Matsu was from a Japanese macaque born in captivity at the South Texas Primate Observatory, whose parents were from a troop which was transported from Arashiyama in Kyoto to the United States in 1972; strain MM39-83 was from a naturally infected rhesus macaque (*Macaca mulatta*) born in captivity in 1978 and maintained at the New England Regional Primate Center since 1983 (Daniel *et al.*, 1988); strain KIA was derived from a yellow baboon (*Papio cynocephalus*) captured in southern Africa and housed separately except for scheduled matings in the Wiseman Hall Vivarium at the Ohio State University in Columbus (Dezzutti *et al.*, 1992); strain Somayer was from a sooty mangabey (*Cercocebus atys*), originally from West Africa, maintained at the Yerkes Regional Primate Research Center in Atlanta. All captive monkeys were housed only with animals of the same species.

Gene amplification and nucleotide sequencing

High-molecular-weight DNA, extracted from virus-infected T-cell lines, was used for gene amplification. Unamplified genomic DNA and enzymatically amplified products were handled by different personnel in separate rooms to minimize carryover. Oligonucleotide primers for PCR and for direct DNA sequencing were derived from gene sequences of the Japanese HTLV-I strain ATK (Seiki *et al.*, 1983) for the amino-terminal p24-encoding region of *gag* (bases 1423–1444, 5′-CCATCACCAGCAGCTAGATAGC-3′ and bases 1560–1537, 5′-AGTTGCTGGTATTCTCGCCTTAAT-3′); the 3′-end of *pol* (bases 4757–4778, 5′-CCCTACAATCCAACCAGCTCAG-3′ and bases 4942–4919, 5′-GTGGTGAAGCTGCCATCGGGTTTT-3′); the amino-terminal gp46-encoding region of *env* (bases 5194–5214, 5′-CCAACACCATGGGTAAGTTTC-3′ and bases 5495–5476, 5′-GCCTCCGCCATTTCGGTTTG-3′; bases 5228–5246, 5′-TTTATTCTTCCAGTTCTGC-3′ and bases 5495–5476, 5′-GCCTCCGCCATTTCGGTTTG-3′); the midportion gp46-encoding region of *env* (bases 5706–5726, 5′-GGATATGACCCCATCTGGCTC-3′ and bases 5992–5973, 5′-GCTGGAAGCGCTAACGATGG-3′); and 5′-end of the orf-II of *pX* (bases 7358–7377, 5′-CGGATACCCAGTCTACGTGT-3′ and bases 7516–7496, 5′-GAGCCGATAACGCGTCCATCG-3′). All primers were used at a final concentration of 1 μM in a reaction mixture of 100 μl containing 1 μg of DNA and 2.5 units of *Thermus aquaticus* DNA polymerase (Perkin–Elmer/Cetus, Norwalk, CT). Mixtures were cycled 45 times, as described previously (Nerurkar *et al.*, 1992). Enzymatically amplified DNA, purified using Centricon-100 microconcentrators (Amicon Division, Danvers, MA), were sequenced in both directions using the *Taq* dye deoxy-terminator cycle sequencing kit (Applied Biosystems Inc., Foster City, CA) on an automated sequencer (Model 373A, Applied Biosystems, Inc.). Sequence ambiguity was resolved by manual sequencing using the Sequenase Version 2.0 DNA sequencing kit (U.S. Biochemicals, Cleveland, OH).

Genetic and phylogenetic analyses

Nucleotide and deduced amino acid sequences of the *gag, pol, env,* and *pX* gene regions for the Asian and African STLV-I strains were aligned and compared

with sequences of other STLV-I and HTLV-I strains from widely separated regions (Table 1), including STLV-I strain PtM3 from a pig-tailed macaque (*Macaca nemestrina*) from Indonesia (Watanabe *et al.*, 1985), strain Tan90 from a tantalus monkey (*Cercopithecus aethiops* var. tantalus) from Central Africa (Saksena *et al.*, 1993), strains AGM22 from a green monkey (*C. aethiops*) from Kenya and ChM114-1 from a common chimpanzee (*Pan troglodytes*) from Sierra Leone (Yanagihara *et al.*, 1993), and viral isolates (designated Sukhumi T-cell lymphoma virus or SuTLV) from sacred or hamadryas baboons (*Papio hamadryas*) maintained at the Sukhumi Primate Center in Georgia (Schätzl *et al.*, 1992), as well as HTLV-I strains ATK (Seiki *et al.*, 1983) and MT-2 (Gray *et al.*, 1989, 1990) from Japan, strain BEL 1 (Nerurkar *et al.*, 1993b) from Bellona, strain CMCH 13 (Nerurkar *et al.*, 1993a) from southern India, strains HS-35 (Malik *et al.*, 1988) and CH (Paine *et al.*, 1991; Ratner *et al.*, 1991) from the Caribbean basin, strain CR (De *et al.*, 1991) from the United States, strain ST (Dekaban *et al.*, 1992) from Chile, strain EL (Paine *et al.*, 1991; Ratner *et al.*, 1991) from equatorial Zaire, strain MEL 1 (Nerurkar *et al.*, 1993b) from Papua New Guinea, and strain MEL 5 (Nerurkar *et al.*, 1993b) from the Solomon Islands.

Sequence alignments were facilitated by using the software package available on the VAX computer system, as part of the Genetics Computer Group (Devereux *et al.*, 1984). Phylogenetic trees, rooted by assuming HTLV-II as the outgroup, were constructed by using the neighbor-joining method (Saitou and Nei, 1987) and the maximum parsimony method (Swofford, 1993), which accommodate variable rates of molecular change. Evolutionary distances (number of nucleotide substitutions) were estimated using the one-parameter (Jukes and Cantor, 1969) and two-parameter methods (Kimura, 1980), and these distances were used for constructing neighbor-joining trees. Bootstrap probabilities, based on 1000 resamplings, were calculated for each internal branch of the neighbor-joining trees using the NJBOOT2 program (kindly provided by Dr. Koichiro Tamura of Tokyo Metropolitan University). PAUP Version 3.1 (Swofford, 1993) was used for maximum parsimony analysis and branch-and-bound search was used to ensure finding the true maximum parsimony trees.

RESULTS

Nucleotide sequence diversity of STLV-I strains

STLV-I strains from Japanese macaque were genetically distinct from cosmopolitan and Melanesian strains of HTLV-I, diverging by 8.8 to 11.2% (Table 2). Compared to the Japanese HTLV-I strain ATK, the Japanese macaque STLV-I strains diverged by 8.7 to 9.8% in the *gag*, 13.6 to 14.3% in the *pol*, 12.4 to 12.8% in the *env*, and 3.4% in the *pX* gene regions (Figs. 1A–E). Overall, the intraspecies nucleotide diversity among the Japanese macaque STLV-I strains ranged from 0.2 to 1.5%. Strain PtM3 isolated from a pig-tailed macaque (*M. nemestrina*) originating from Indonesia showed 98.1 to 98.7 and 98.3% sequence similarity in the *env* and *pX* gene regions, respectively, with the Japanese macaque STLV-I strains, while strain MM39-83 from a rhesus macaque was genetically more different, exhibiting sequence similarities of 83.0 to 84.3 and 92.4%, respectively, in these gene regions.

Compared to the Asian STLV-I strains, the African STLV-I strains were genetically more closely related to cosmopolitan HTLV-I strains, showing sequence similarities of 97.8 to 98.9% in the *gag*, 97.9 to 98.6% in the *pol*, 96.1 to 96.9% in the *env*, and 97.5 to 98.3% in the *pX* gene regions with the Zairian HTLV-I strain EL. A similar degree of sequence similarity was found among the African STLV-I strains when compared to the Japanese HTLV-I strain ATK (Table 2). Moreover, despite originating from four genera of nonhuman primates, the interspecies nucleotide sequence similarity among the African STLV-I strains was remarkably high, ranging from 96.9 to 97.4% overall in the four gene regions.

A region of sequence variability was found at positions 5252 to 5266 in the 5′-end of the gp46-encoding region of the *env* gene; base substitutions in this region were more abundant among the Asian STLV-I strains and the Melanesian HTLV-I strains than among African STLV-I strains and cosmopolitan HTLV-I strains (Fig. 1C). Similarly, in the highly conserved region of the 5′-end of *pX*-II, the Asian STLV-I strains were more variable (four to six substitutions) than the African STLV-I strains (zero to three substitutions), with the exception of strain Tan90 from a tantalus monkey from the Central African Republic (four substitutions; Fig. 1E). The *pX*-II sequence of strain MM39-83 was identical to that of strain SuTLV from captive hamadryas baboons housed at the Sukhumi Primate Center, but otherwise nearly all of the base substitutions were not shared between Asian and African STLV-I strains.

Of the 59 to 69 base substitutions in the *gag*, *pol*, *env*, and *pX* gene regions of the STLV-I strains from Japanese and rhesus macaques, 84 to 92% were transitions, predominantly deoxycytosine to deoxythymidine or vice versa. Moreover, 14 to 23% of the base substitutions occurred at codon position 1, 6 to 7% at codon position 2, and 70 to 80% at codon position 3. Similarly, among the African STLV-I strains, transitions accounted for 88 to 91% of all base changes, of which 76 to 82% occurred at codon position 3.

Deduced amino acid sequence analysis of STLV-I strains

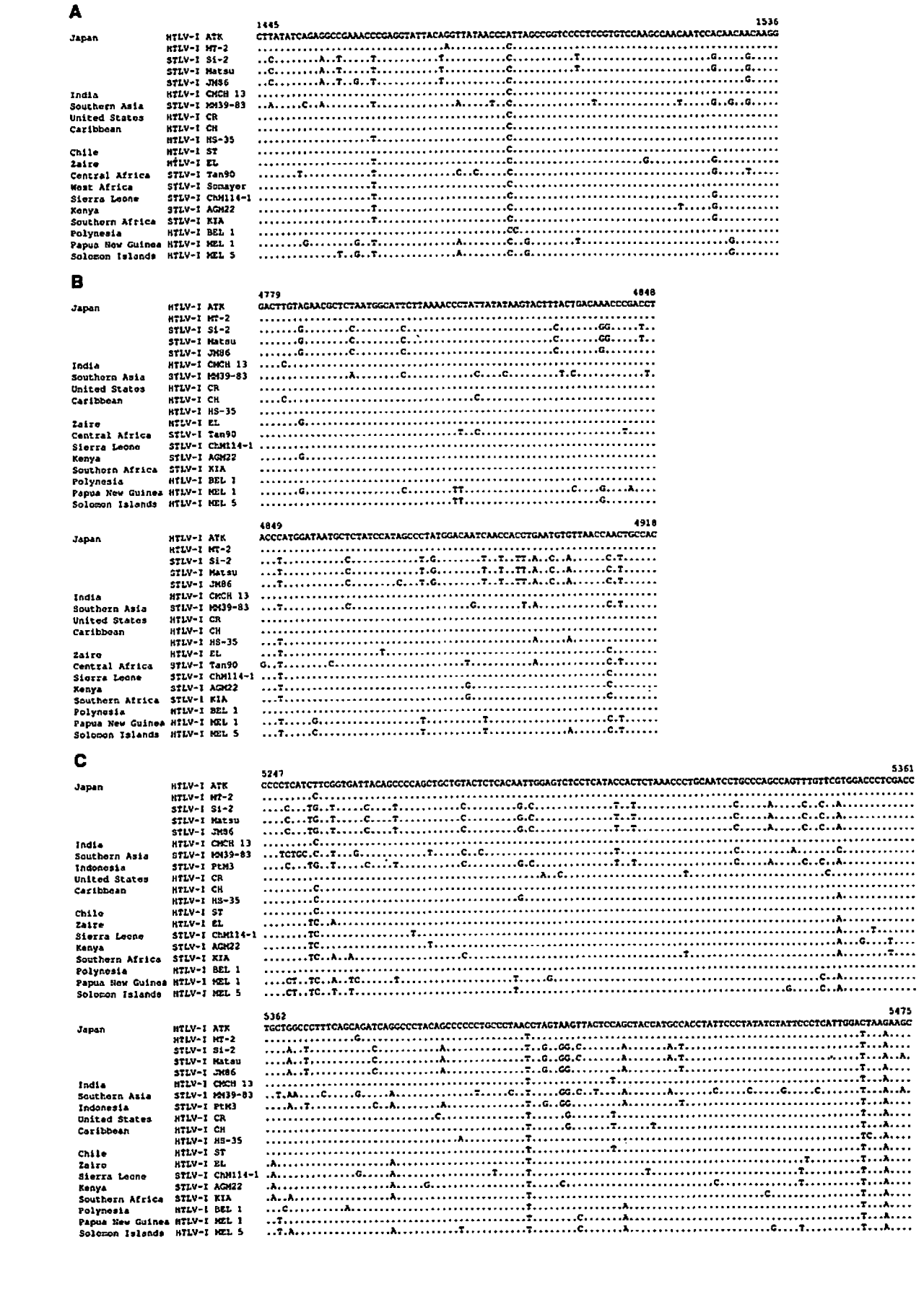
All base substitutions in the 31-amino acid p24 capsid-encoding region of the *gag* gene of the Asian and

TABLE 2

NUCLEOTIDE AND AMINO ACID HOMOLOGIES BETWEEN HTLV-I AND STLV-I ISOLATES FROM VARIOUS GEOGRAPHIC AREAS

Strain	Strain																
	HTLV-I ATK	HTLV-I CMCH 13	HTLV-I BEL 1	HTLV-I MT-2	HTLV-I CH	HTLV-I CR	HTLV-I HS-35	HTLV-I EL	STLV-I KIA	STLV-I ChM114-1	STLV-I AGM22	HTLV-I MEL 5	HTLV-I MEL 1	STLV-I JM86	STLV-I Si-2	STLV-I Matsu	STLV-I MM39-83
	Percentage homology of nucleotide and amino acid sequences																
HTLV-I ATK		99.0	98.4	97.9	97.9	96.9	98.4	97.4	97.4	97.9	97.4	95.3	94.8	94.8	94.2	94.2	92.2
HTLV-I CMCH 13	98.8		98.4	99.0	99.0	96.9	99.5	98.4	98.4	99.0	98.4	96.3	95.8	95.3	94.8	94.8	93.2
HTLV-I BEL 1	98.6	98.8		97.4	97.4	96.3	97.9	96.9	96.9	97.4	96.9	94.8	94.2	94.2	93.7	93.7	91.6
HTLV-I MT2	98.6	99.1	98.6		97.9	95.8	98.4	97.4	97.4	97.9	98.4	95.3	94.8	94.2	93.7	93.7	92.2
HTLV-I CH	98.3	99.5	98.3	98.6		96.9	98.4	97.4	97.4	97.9	97.4	95.3	94.8	95.3	94.8	94.8	93.2
HTLV-I CR	98.1	98.6	98.1	98.1	98.5		96.3	95.3	95.3	95.8	95.3	93.2	92.7	93.7	93.2	93.2	91.1
HTLV-I HS-35	97.8	98.3	97.8	98.1	97.8	97.2		97.9	97.9	98.4	97.9	95.8	95.3	95.8	95.3	95.3	92.7
HTLV-I EL	96.6	97.1	96.6	96.9	96.6	96.0	97.1		99.0	98.4	97.9	96.3	96.3	95.3	94.8	94.8	93.2
STLV-I KIA	96.4	97.0	96.4	96.7	96.4	96.2	97.0	97.8		98.4	97.9	96.9	96.9	95.3	94.8	94.8	93.7
STLV-I ChM114-1	96.2	96.7	96.2	96.9	96.2	95.7	96.7	97.6	97.1		98.4	96.3	95.8	95.3	94.8	94.8	93.2
STLV-I AGM22	95.9	96.4	95.9	96.6	95.9	95.3	96.4	97.2	97.4	96.9		95.8	95.3	94.8	94.2	94.2	92.7
HTLV-I MEL 5	93.4	94.0	93.8	93.8	93.4	92.9	94.5	94.0	94.3	93.8	93.4		97.4	96.9	96.3	96.3	93.7
HTLV-I MEL 1	93.1	93.6	93.4	93.4	93.1	92.6	93.6	93.8	93.4	92.8	92.8	96.6		95.8	95.3	95.3	92.7
STLV-I JM86	89.8	90.2	90.2	90.0	90.0	89.6	91.2	90.2	90.3	89.3	89.5	90.7	90.2		99.5	99.5	93.2
STLV-I Si-2	89.1	89.5	89.5	89.3	89.3	89.0	90.5	89.8	90.0	89.0	89.1	90.0	89.8	98.6		100	93.7
STLV-I Matsu	89.0	89.3	89.3	89.1	89.1	88.8	90.3	89.6	89.8	88.8	89.0	89.8	89.6	98.5	99.8		93.7
STLV-I MM39-83	88.1	88.6	88.4	88.8	88.8	87.9	88.8	88.8	89.0	88.6	88.8	88.3	88.1	86.7	87.1	87.2	

Note. Pairwise homologies based on 579-bp sequences of the *gag*, *pol*, *env*, and *pX* genes are presented as a triangular matrix. Nucleotide homologies are presented in the lower half and amino acid homologies in the upper half (estimated by DISTANCE).


A
1445
1536
Japan HTLV-I ATK CTTATATCAGAGGCCGAAACCCGAGGTATTACAGGTTATAACCCATTAGCCGGTCCCCTCCGTGTCCAAGCCAACAATCCACAACAACAAGG
HTLV-I MT-2
STLV-I Si-2
STLV-I Matsu
STLV-I JM86
India HTLV-I CMCH 13
Southern Asia STLV-I MM39-83
United States HTLV-I CR
Caribbean HTLV-I CH
HTLV-I HS-35
Chile HTLV-I ST
Zaire HTLV-I EL
Central Africa STLV-I Tan90
West Africa STLV-I Sonayer
Sierra Leone STLV-I ChM114-1
Kenya STLV-I AGM22
Southern Africa STLV-I KIA
Polynesia HTLV-I BEL 1
Papua New Guinea HTLV-I MEL 1
Solomon Islands HTLV-I MEL 5
B
4779
4848
Japan HTLV-I ATK GACTTGTAGAACGCTCTAATGGCATTCTTAAAACCCTATTATATAAGTACTTTACTGACAAACCCGACCT
4849
4918
Japan HTLV-I ATK ACCCATGGATAATGCTCTATCCATAGCCCTATGGACAATCAACCACCTGAATGTGTTAACCAACTGCCAC
C
5247
5361
Japan HTLV-I ATK GCCCTCATCTTCGGTGATTACAGCCCCAGCTGCTGTACTCTCACAATTGGAGTCTCCTCATACCACTCTAAACCCTGCAATCCTGCCCAGCCAGTTTGTTCGTGGACCCTCGACC
5362
5475
Japan HTLV-I ATK TGCTGGCCGTTTCAGCAGATCAGGCCCTACAGCCCCCCTGCCCTAACCTAGTAAGTTACTCCAGCTACCATGCCACCTATTCCCTATATCTATTCCCTCATTGGACTAAGAAGC

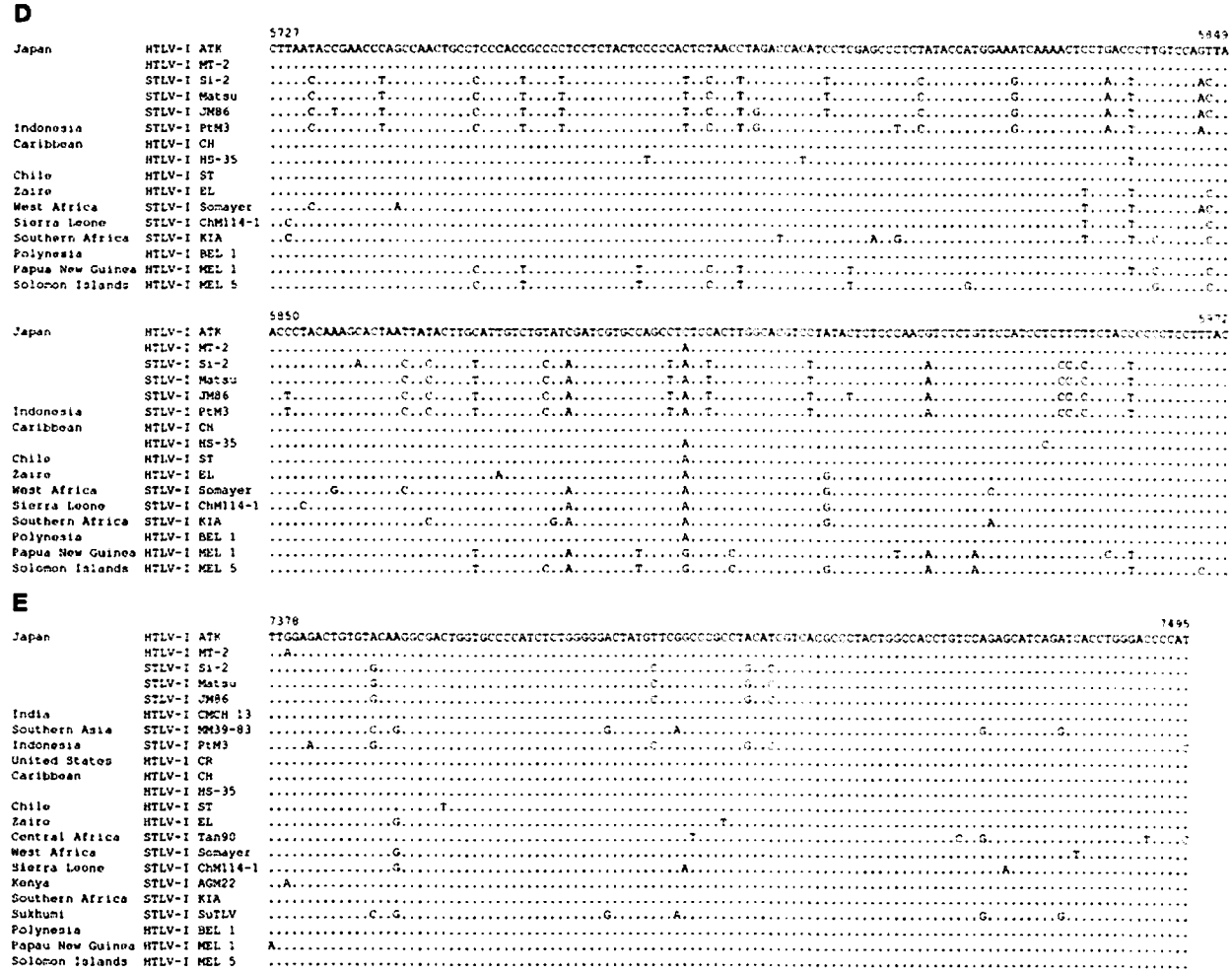


FIG. 1. Alignment and comparison of nucleotide sequences of (A) the p24-encoding region of the *gag* gene (bases 1445 to 1536), (B) the 3'-end of the *pol* gene (bases 4779 to 4918), (C) the 5'-end of the gp46-encoding region of the *env* gene (bases 5247 to 5475), (D) the neutralizing epitope-spanning, midportion of the gp46-encoding region of the *env* gene (bases 5727 to 5972), and (E) the tax-encoding region of the *pX* gene (bases 7378 to 7495) in STLV-I strains from Asian (Si-2, Matsu, JM86, MM39-83, PtM3) and African (AGM22, Tan90, KIA, Somayer, ChM114-1) catarrhines. For comparison, corresponding sequences are shown for HTLV-I strains from Japan (ATK, MT-2), India (CMCH 13), the Caribbean (CH, HS-35), the United States (CR), Chile (ST), Zaire (EL), the Polynesian Outlier Bellona (BEL 1), Papua New Guinea (MEL 1), and the Solomon Islands (MEL 5). The asterisks represent deletions. Nucleotide sequences for STLV-I strains have been deposited into GenBank under Accession Nos. L20662, L20663, L20664, L20665, and L20666 for Si-2; L20653, L20654, L20655, L20656, and L20657 for Matsu; L20643, L20644, L20645, L20646, and L20647 for JM86; L20658, L20659, L20660, and L20661 for MM39-83; L20648, L20649, L20650, L20651, and L20652 for KIA; and L20667, L20668, and L20669 for Somayer.

African STLV-I strains were silent, while in the 46-amino acid 3'-end of the *pol* gene, some unique amino acid changes were found. Compared to the Japanese HTLV-I strain ATK, the STLV-I strains from Japanese macaques had two amino acid-altering base substitutions at positions 4839 (Lys to Arg) and 4910 (Asp to His) and strain MM39-83 from rhesus macaque had three such mutations at positions 4832 (Thr to Ser), 4886 (Ile to Val), and 4910 (Asp to His). STLV-I strains from Africa, like HTLV-I strains from Zaire and Melanesia, but unlike HTLV-I strains from Japan, India, the Caribbean, and the Americas, had the same base substitution at position 4910.

Multiple amino acid substitutions were found in the amino-terminal gp46-encoding region of the *env* gene: STLV-I strains from Japanese and rhesus macaques differed by 8 to 9 (10.5 to 11.8%) amino acids and by 11 (14.5%) amino acids, respectively, when compared to HTLV-I strain ATK. African STLV-I strains from green monkey, yellow baboon, and chimpanzee were considerably less variant, diverging by 2 to 4 (2.6 to 5.3%) amino acids from HTLV-I strain ATK and by 2 to 3 (2.6 to 3.9%) amino acids from HTLV-I strain EL. In the region of the signal peptide, the deduced sequences for amino acids 17 to 22 were Pro*Ile**Val**Cys*Asp*His* for STLV-I strains Si-2, Matsu, JM86, and PtM3; *Ser**Ala**Leu**Cys**Gly*Tyr for STLV-I strain MM39-83; Leu*Ile**Leu**Ser**Asn*Tyr for STLV-I strain KIA; Leu*Ile**Leu*GlyAspTyr for STLV-I strains AGM22 and ChM114-1 and for HTLV-I strains MT-2, CMCH 13, CH, HS-35, and ST;

LeuIle*LeuSer*AspTyr for HTLV-I strain EL; *Pro*Ile*LeuSerSer*Tyr for HTLV-I strain MEL 1; and *Pro*Ile*LeuCysTyr*Tyr for HTLV-I strain MEL 5, compared to LeuIlePheGlyAspTyr for HTLV-I strains ATK, BEL 1, and CR (different residues are italic).

Considerable sequence conservation of the neutralizing domains, composed of amino acids 88 to 98 (TrpIleLysLysProAsnArgAsnGlyGlyGly) and 191 to 196 (LeuProHisSerAsnLeu) on the amino-terminal and the midportion of the gp46 external envelope glycoprotein, respectively, was found in Asian and African strains of STLV-I and in HTLV-I from widely separated geographic regions, suggesting that these simian and human type C retroviruses may all exist as a single serotype.

The *tax*-reading frame was well conserved in the Asian and African STLV-I strains, with either no or just one amino acid change compared to HTLV-I strain ATK. By contrast, in the *pX*-II and *rex*-reading frames, the Japanese and rhesus macaque STLV-I strains had four to six amino acid changes, and in the *pX*-II region, the African STLV-I strains differed by two or three residues from HTLV-I strain ATK or exhibited a stop codon.

Phylogenetic relationship between STLV-I and HTLV-I strains

Unrooted phylogenetic trees based on *gag* (92 bp) and *pol* (140 bp) gene sequences and on *gag* (92 bp), *pol* (140 bp), *env* (229 bp), and *pX* (118 bp) gene sequences of STLV-I and HTLV-I strains were constructed using the neighbor-joining method (Figs. 2A and 2B) and the maximum parsimony method (data not shown). Neighbor-joining trees for each data set, obtained from evolutionary distances based on one- and two-parameter methods and rooted by assuming HTLV-II as the outgroup, were identical in their branching patterns or topologies, and branch lengths were nearly the same. By the maximum parsimony method, 16 equally parsimonious trees, requiring 167 nucleotide (nt) substitutions, were constructed from the 232-bp *gag* and *pol* gene sequences. The corresponding neighbor-joining tree (Fig. 2A) was not a maximum parsimonious one, requiring two additional (or 169) nucleotide substitutions. Of the 12 equally parsimonious trees based on the 579-bp *gag*, *pol*, *env*, and *pX* gene sequences, which required 375 nt substitutions, one had an identical branching pattern with the neighbor-joining tree (Fig. 2B).

In the neighbor-joining tree based on 579 nt sites, bootstrap probabilities (in percentage), as determined for 1000 resamplings by NJBOOT2, for the branches clustering the STLV-I strains from Japanese macaques, the Melanesian HTLV-I strains, and the cosmopolitan HTLV-I strains, including STLV-I strains from Africa, were extremely high (99 to 100%), demonstrating that the Asian and African STLV-I strains have evolved independently (Fig. 2B). The smaller number of nucleotide sites probably accounted for the somewhat lower bootstrap probabilities for these clusters in the neighbor-joining tree based on the 232-bp *gag–pol* gene sequences (Fig. 2A). No HTLV-I strain was closely related to any Asian STLV-I strain, whereas a bootstrap probability of 84% was found for the cluster formed by African STLV-I strains AGM22, KIA, and ChM114-1 and the HTLV-I variant EL from equatorial Zaire (Fig. 2B).

The overall congruency of the phylogenetic trees, based on 232 and 579 nt sites using the neighbor-joining and maximum parsimony methods, validated the evolutionary relationship among the African and Asian STLV-I strains and the Melanesian and cosmopolitan strains of HTLV-I. The only conspicuous discrepancy between trees based on the 232-bp *gag–pol* sequences, constructed by the neighbor-joining and maximum parsimony methods, was the positioning of STLV-I strain Tan90 from Central Africa. In the neighbor-joining tree, strain Tan90 clustered with the African STLV-I and cosmopolitan HTLV-I strains, but the bootstrap probability was low (51%; Fig. 2A). By contrast, strain Tan90 fell outside all STLV-I and HTLV-I strains in the strict consensus tree for the 16 equally parsimonious trees (not shown). However, the bootstrap probability for this cluster (HTLV-II and Tan90) was only 39%. More sequence information is needed to more precisely determine the phylogenetic position of STLV-I strain Tan90.

DISCUSSION

As evidenced by similarities in their genomic organization and biological properties, HTLV-I and STLV-I are closely related oncogenic retroviruses. Therefore, sequence analysis of STLV-I strains is useful not only to determine the genetic diversity and molecular evolution of STLV-I but also to clarify their phylogenetic relationship to HTLV-I. The overall paucity of sequence information on STLV-I strains from various catarrhine hosts in widely separated geographical regions make any discussion about the evolution of STLV-I tentative and speculative, but we offer the following observations.

The close genetic relationship between STLV-I strains from Japanese and pig-tailed macaques, previously inferred from their restriction maps (Watanabe *et al.*, 1986), was verified by our sequence data. By contrast, strain MM39-83 from a rhesus macaque was genetically distant, diverging by more than 10% from STLV-I strains from Japanese macaques and African catarrhines. Additional studies are required to verify if strain MM39-83 is truly representative of STLV-I

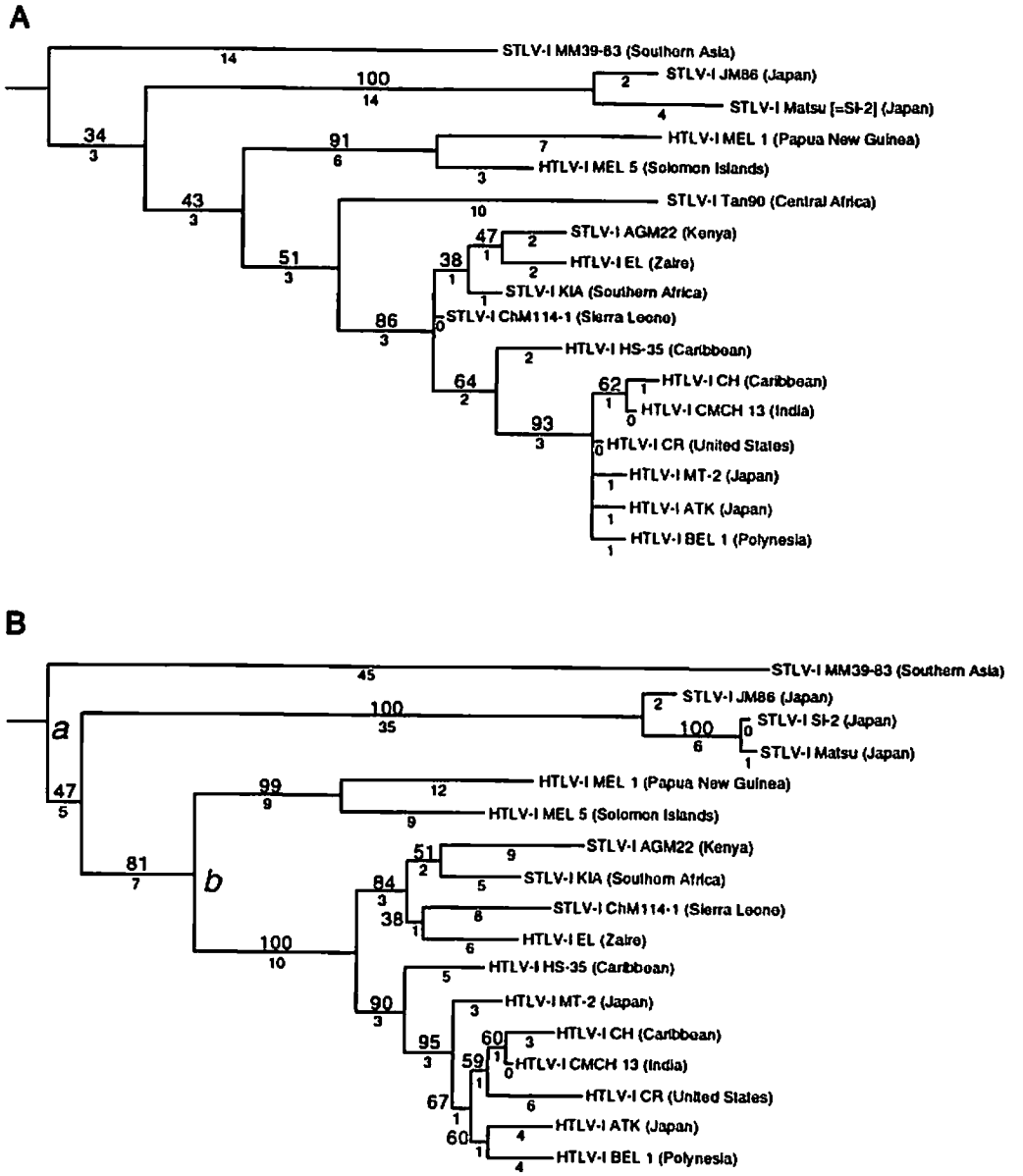


FIG. 2. Phylogenetic trees based on (A) the 92-bp region of the p24-encoding *gag* gene and the 140-bp region of the *pol* gene and (B) the 579 bp from selected regions of the *gag, pol, env,* and *pX* genes of Asian and African STLV-I strains and corresponding sequences of HTLV-I strains from various geographical areas, constructed by the neighbor-joining method (see text for details). The trees were rooted by assuming HTLV-II (Shimotohno *et al.*, 1985) as the outgroup. Branch lengths, given below each branch, are proportional to the estimated number of nucleotide substitutions, and bootstrap probabilities (in percentage), as determined for 1000 resamplings by NJBOOT2, are given above or beside the internal branches. Sequences of the *gag–pol* region were identical for STLV-I strains Matsu and Si-2 from Japanese macaques. The unavailability of *gag* and *pol* sequences for STLV-I strain PtM3 from an Indonesian pig-tailed macaque did not permit its inclusion in these trees. Similarly, DNA was insufficient for STLV-I strain Somayer for amplification and sequencing of the *pol* gene region. Point *a* indicates the divergence between STLV-I strains from rhesus and Japanese macaques and point *b* denotes the time of human occupation of Melanesia (see text for details).

strains from rhesus macaque because of the wide geographical range of rhesus monkeys in southern Asia (Wolfheim, 1983), extending from Afghanistan to Pakistan and throughout much of southeast Asia including the eastern coast of China (as well as the Indo–Malay peninsula where rhesus monkeys are sympatric and synchronistic with pig-tailed macaques and stump-tailed macaques).

Despite their wide host range, the STLV-I strains from Africa exhibited a remarkably close genetic relationship among themselves and tended to segregate with HTLV-I strain EL from equatorial Zaire, suggesting that in certain instances the designation HTLV-I and STLV-I may be semantic. In this regard, recent genetic analysis indicates that human immunodeficiency virus type 2 and simian immunodeficiency virus from wild

sooty mangabeys in West Africa are probably the same virus (Gao *et al.*, 1992). By contrast, STLV-I strains from Japanese and rhesus macaques were genetically quite distant from HTLV-I strains from Japan and India. Thus, the HTLV-I strains in Japan and India could not have evolved recently from STLV-I from Japanese and rhesus macaques. Several possible explanations may account for the regional genetic differences between STLV-I and HTLV-I in Asia and Africa. First, interspecies virus transmission between nonhuman primates (and possibly from nonhuman primates to humans) may have occurred more frequently in Africa than in Asia. Second, the common ancestor of STLV-I may have emerged outside of Africa, with subsequent independent virus evolution in Asia and Africa. Third, the rate of molecular change for STLV-I among Asian and African nonhuman primates may not be constant or similar. Fourth, as in Africa, virus variants that genetically link STLV-I and HTLV-I may exist in Asia.

Based on our sequence data, the origin of the recently reported virus isolates, designated SuTLV (Schätzl *et al.*, 1992), from "lymphoma-prone" sacred or hamadryas baboons (*P. hamadryas*) housed at the Sukhumi Primate Center in Georgia of the former Soviet Union, and their phylogenetic relationship with other STLV-I strains are unclear. If SuTLV is an indigenous baboon virus, it is genetically quite distant from strain KIA isolated from a yellow baboon (*P. cynocephalus*) and from STLV-I strains from other African nonhuman primates. Thus, SuTLV would be the most divergent African STLV-I identified to date. On the other hand, the close genetic relationship between SuTLV and strain MM39-83 from a rhesus macaque suggests that SuTLV from hamadryas baboons may be the phylogenetic link between STLV-I strains from African and Asian catarrhines. Alternatively, SuTLV may have originated from interspecies virus transmission from rhesus macaques. This latter possibility is supported by our preliminary sequence analysis of STLV-I strains from other naturally infected baboons which indicates a high degree of sequence similarity with strain KIA (unpublished observations). Moreover, serological surveys conducted among wild-captured hamadryas baboons in Ethiopia indicate no evidence of STLV-I infection, whereas antibodies against STLV-I are frequently found among anubis baboons (*Papio anubis*) and hybrid offspring of anubis and hamadryas baboons (Yamamoto *et al.*, 1988).

As judged by morphological features and genetic distance analysis, *M. fuscata* (Japanese macaque), *M. mulatta* (rhesus macaque), *M. cyclopis* (Formosan rock macaque), and *M. fascicularis* (crab-eating macaque) are very closely related, with *M. cyclopis* being essentially a race of *M. mulatta* and *M. mulatta* being the closest genetic relative of *M. fuscata* (Melnick and Kidd, 1985; Melnick *et al.*, 1993). Paleontological data, blood protein polymorphisms and restriction-enzyme and sequence analyses of mitochondrial DNA indicate that Japanese and rhesus macaques diverged 0.3 to 1.8 million years ago (Hayasaka *et al.*, 1988; Melnick and Kidd, 1985). Recent reanalysis of mitochondrial DNA sequences from rhesus monkeys from Pakistan, India, Burma, and China indicates that the higher figure given above may represent an overestimation and that the lower figure may be more accurate (Melnick *et al.*, 1993). However, if this divergence time corresponds to the branching point between STLV-I strain MM39-83 from rhesus macaque and strains JM86 and Matsu from Japanese macaque (point *a* in Fig. 2B), the rate of molecular change for the 579 nt spanning selected regions of the *gag*, *pol*, *env*, and *pX* genes becomes $0.4–2.5 \times 10^{-7}$ substitutions per site per year, given the evolutionary distance of 0.15 (estimated by the two-parameter method) between the rhesus and Japanese macaque STLV-I strains. Because the branch lengths from branch *a* to each strain are more or less the same, a similar evolutionary rate applies to HTLV-I. If so, the divergence time for point *b* in Fig. 2B is estimated to be 140,000 to 850,000 years before present, since the evolutionary distance between Melanesian and cosmopolitan strains, estimated by the two-parameter method, is approximately 0.068. In this instance, virus transmission from macaques to humans must have occurred at a relatively early stage of modern human evolution.

Alternatively, if we assume, based on the archaeological record of human occupation in Melanesia, that the branching point *b* in Fig. 2B is 50,000 years ago, then the rate of genetic change for the 579-bp region of HTLV-I becomes 6.8×10^{-7} substitutions per site per year, and the divergence time for point *a* in Fig. 2B becomes 110,000 years before present. Since the time of geographical separation between Japan and mainland Asia occurred after the last glaciation approximately 11,000 years ago (Melnick and Kidd, 1985), virus transmission between Japanese and rhesus macaques must have occurred before that time. Irrespective of which of these estimates is more accurate, the collective data are consistent with an archaic presence of STLV-I in Asia, probably predating macaque speciation, with interspecies transmission of the common ancestral strain of STLV-I long before the present-day habitats of their primate hosts were established in Africa and Asia.

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