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Isolation and Characterization of Simian T-Cell Leukemia Virus Type II from New World Monkeys

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Since the description of human T-cell leukemia virus type I (HTLV-I) and its simian counterpart, simian T-cell leukemia virus type I (STLV-I), the possible existence of other related simian retroviruses has been raised. Here, we report a new retrovirus, STLV-II, which we have identified in spider monkeys (*Ateles fusciceps*), a New World primate species. Initially, a recombinant HTLV-II envelope protein (RP-IIB) was used to identify anti-STLV-II antibodies in New World monkeys by Western blot (immunoblot) assays. Subsequently, the virus was characterized by Southern blot hybridization, which showed that STLV-II and HTLV-II have a high degree of nucleotide sequence homology but have different restriction enzyme patterns. Nucleotide sequence analysis of the *pX*-II region of STLV-II provirus revealed 3% variation with the corresponding region of HTLV-II. Electron micrographic studies revealed HTLV-like, type C retrovirus particles outside the cell membranes of STLV-II-infected cells. This study describes the first link between HTLV-II and a simian reservoir in the New World. Further molecular studies of STLV-II infection in different species of New World monkeys, especially from the wild, may provide valuable information about the origin and intragroup relationships of South American monkeys. Spider monkeys infected with STLV-II may serve as an important animal model for HTLV-II infection in humans.

Human T-cell leukemia viruses (HTLVs) are exogenous retroviruses and belong to the same *Oncovirinae* subfamily of the *Retroviridae* as bovine leukemia virus and simian T-cell leukemia virus (STLV) (53). HTLV type I (HTLV-I) was first isolated in 1980 from a T-lymphoblastoid cell line established from a patient with cutaneous T-cell lymphoma (38). It has been found to be associated with adult T-cell leukemia/lymphoma (55, 60) and HTLV-I-associated myelopathy/tropical spastic paraparesis (12, 37). HTLV-II was first isolated from a patient with atypical hairy cell leukemia (27). Although there have been scattered case reports, the disease associated with HTLV-II infection is still unclear (43). On the basis of nucleic acid hybridization analysis, direct comparison of primary nucleic acid sequences of long terminal repeats, and the extent of serologic cross-reactivity between *gag* gene-encoded products, HTLV-II has been shown to be related but distinct from HTLV-I (3, 48, 52). In general, HTLV-I and HTLV-II have approximately 65% genomic homology (46, 49).

In 1982, Miyoshi and colleagues identified HTLV-related viruses in nonhuman primates by demonstrating the presence of anti-HTLV antibodies in Japanese macaques (*Macaca fuscata*) (36). Since then, many studies have found antibody reactivities to HTLV in different species of the genus *Macaca* as well as in many African species of Old World primates (17, 18, 22, 24, 26, 35, 59). Furthermore, a serological survey of captive macaques from Asia indicated strong association of lymphoma and lymphoproliferative disease with STLV infection (22). Nucleotide sequence analysis of an STLV isolate from pig-tailed macaques revealed 90% homology with cosmopolitan strains of HTLV-I (57). Recently, serological tests have also shown that STLV infections in Old World primates are mainly due to STLV type I (STLV-I) (44).

The recent identification of type-specific regions on the envelope glycoproteins of HTLV-I and HTLV-II (7, 8, 30, 31) and development of serological tests complementary to DNA diagnosis by PCR have enabled a clarification of the geographic and racial distributions of the two viruses; HTLV-I infections are endemic in regions of Africa, the Caribbean islands, and southern islands of Japan (2, 19, 45), while HTLV-II is endemic in several Indian tribes in Central and South America (20, 29). Since it has been postulated that HTLV-I infections in humans may originate from cross-species transmission through close contact with STLV-I-infected primates, we used an HTLV-II recombinant envelope protein (RP-IIB) (5) to identify HTLV-II-related virus (STLV-II) infection in various species of New World monkeys by Western blot (immunoblot) assays.

MATERIALS AND METHODS

New World monkeys and blood samples. Serum samples from the following numbers of various species of New World monkeys were used in this study: 9 spider monkeys (*Ateles fusciceps*; designated SM-1 to SM-9), 47 owl monkeys (*Aotus*

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trivirgatus), 10 capuchin monkeys (*Cebus albifrons*), 20 howler monkeys (*Alouatta villosa*), 2 squirrel monkeys (*Saimiri sciureus*), and 18 marmosets (2 *Callithrix jacchus*, 12 *Saguinus oedipus*, and 4 *Saguinus flaviceps*). All of the monkeys were captive and were housed at primate centers or zoos. The howler monkeys were caught in the wild, and their blood was later sampled in Venezuela.

Cell lines and cultures. For virus isolation, several human cell lines, including a T-cell line (Sup-T1) (32, 51), an Epstein-Barr virus immortalized B-cell line (BJAB) (15), and a promonocyte line (THP-1) (54), were used in this study. An HTLV-I-producing cell line (HUT-102) (40) and an HTLV-II-producing cell line [729(pH6neo)] (4) were also used as positive HTLV controls. The cell lines were passaged in RPMI 1640 medium (GIBCO-BRL, Gaithersburg, Md.) supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 IU of penicillin per ml, and 100 IU of streptomycin per ml.

Virus isolation and cell immortalization. Peripheral blood mononuclear cells (PBMCs) from anti-RP-IIB antibody-positive spider monkeys were collected, stimulated with phytohemagglutinin-L, and cultured in the presence of interleukin-2. Ten days later, the cells were cocultivated with human cell lines for virus isolation as described previously (34). For the cell immortalization study, human cord blood lymphocytes were purified by lymphocyte separation medium (Organon Teknika Corporation, Durham, N.C.) and incubated in RPMI 1640 medium containing 20% fetal calf serum and 10 μg of phytohemagglutinin-L (Sigma, St. Louis, Mo.) per ml. Three days later, recombinant human interleukin-2 (Genzyme, Cambridge, Mass.) was added to the culture at a concentration of 100 U/ml. The cord blood lymphocytes were then cocultivated with STLV-II-infected monkey PBMCs which had been subjected to 3,000 rad of radiation.

HTLV-I and HTLV-II recombinant proteins. Two recombinant proteins (RP-B1 and RP-IIB) described previously were used to analyze the antibody reactivities of monkey sera by Western blot assays (5, 6). RP-B1 contains amino acid residues 166 to 201 of the HTLV-I exterior glycoprotein gp46, and RP-IIB contains amino acid residues 96 to 235 of HTLV-II exterior glycoprotein gp46.

Western blot analysis. Detailed procedures have been described previously (8). To detect monkey antibodies specific to HTLV-I and/or -II recombinant proteins, a 1/100 dilution of monkey serum samples and a 1/100 dilution of biotinylated sheep anti-human immunoglobulin (Amersham, Arlington Heights, Ill.) were used in the assay. For each test panel, HTLV-I- or HTLV-II-positive serum at a 1/200 dilution was used as the positive control.

PCR. For the PCR assay, conditions were as recommended by the manufacturer (Perkin-Elmer Cetus), except that the concentration of $MgCl_2$ was 2 mM (1). Thirty-two cycles of amplification with Amplitaq *Taq* DNA polymerase were performed in a Perkin-Elmer Cetus DNA thermal cycler. Two micrograms of lymphocyte DNA from each subject was used as template. Each PCR cycle employed a primer-annealing step at 54°C for 1 min and an extension step at 72°C for 1 min. The nucleotide sequences of primers used in the assay were as follows: SK43, 5′-CGGATACCCAGTCTACGTGT (7358 to 7377 of the HTLV-I *tax* gene); SK44, 5′-GAGCCGATA ACGCGTCATCG (7516 to 7496 of the HTLV-I *tax* gene); SK58, 5′-ATCTACCTCCACCATGTCCG (4198 to 4217 of the HTLV-II *pol* gene); SK59, 5′-TACGGGGAACAAGGG GAGCT (antisense of 4281 to 4300 of the HTLV-II *pol* gene); SK110, 5′-CCCTACAATCCCACCAGCTCAG (4757 to 4778 of the HTLV-I *pol* gene); SK111, 5′-GTGGTGGATTTGCC ATCGGGTTTT (4942 to 4919 of the HTLV-I *pol* gene). Both SK43-SK44 and SK110-111 are HTLV-I and -II generic primers. SK58-SK59 is an HTLV-II-specific primer pair (10).

Dot blot assay. For the dot blot assay, 27 μl of PCR-amplified product was added to a 96-well microtiter plate which contained 3 μl of 3 M NaOH per well, and the plate was incubated at 55°C for 1 h. After denaturation, 30 μl of 2 M ammonium acetate was added to each well and the DNA was transferred to a nitrocellulose membrane by Hybri-Dot 96-well Filtration Manifold (Bio-Rad Laboratories, Hercules, Calif.). ^{32}P-labeled oligoprobes prepared by T4 polynucleotide kinase were used for the hybridization reaction. The nucleotide sequences of the probes used in this assay were as follows: SK45 (an HTLV-I- and/or -II-generic probe), 5′-AGCC CCTACTGGCCACCTGTCCAGAGCATCAGATCACCTG (7447 to 7468 of the HTLV-I *tax* gene), SK60 (an HTLV-II-specific probe), 5′-TAAGGGAGTCTGTGTATTCATTGAA GG TGGAAATTGGGTC (4237 to 4276 of the HTLV-II *pol* gene), SK112 (an HTLV-I-specific probe), 5′-GTACTTTACT GACAAACCCGACCTAC (4825 to 4850 of the HTLV-I *pol* gene), SK188 (an HTLV-II-specific probe), and 5′-TCATGAA CCCCAGTGGTAA (4880 to 4898 of the HTLV-II *pol* gene) (10).

Southern blot hybridization. For Southern blot hybridization, 20 μg of genomic DNA from BJAB cells cocultivated with SM-3 PBMCs, 729(pH6neo), or HUT-102 was digested with various restriction enzymes at 37°C overnight and then electrophoresed in a 0.8% agarose gel at 10 V. The gel was depurinated with 0.25 N HCl for 15 min at room temperature and then denatured (0.5 N NaOH, 1.5 M NaCl) for 1 h and neutralized (1 M Tris-HCl [pH 8.0], 1.5 M NaCl) for 1 h. The gel was then transferred to a nitrocellulose membrane with 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 36 h. After the transfer, the nitrocellulose paper was cross-linked with a UV cross-linker (Spectrolinker X-1000; Spectronics Co., Westbury, N.Y.). The nitrocellulose membrane was then prehybridized with 15 ml of prehybridization solution (5× SSC, 0.1% sodium dodecyl sulfate [SDS], 10 mM Tris-HCl [pH 7.5], 2.5× Denhardt's solution, 100 μg of salmon sperm DNA per ml, 50% deionized formamide, and 10% dextran sulfate) in the hybridization bag and submerged in a water bath at 37°C for 2 h. Two plasmid DNAs, pH6neo (4) and pMT2 (11), which contain full-length HTLV-II or HTLV-I provirus, respectively, were used as probes in the Southern blot hybridization. ^{32}P-labeled probes were prepared with a random primer labeling kit (Promega Co., Madison, Wis.). The ^{32}P-labeled probe was injected into the prehybridization bag, and the bag was incubated in a 37°C water bath for 16 h. The nitrocellulose membrane was then washed with the following: 5× SSC–0.1% SDS at room temperature for 15 min (once) and at 37°C for 15 min (once); 0.2× SSC–0.1% SDS at 37°C for 15 min (four times), at 42°C for 15 min (twice), at 45°C for 15 min (twice), and at 50°C for 15 min (twice); and, finally, 0.1× SSC–0.1% SDS at 55°C for 15 min (twice). The resultant nitrocellulose membrane was exposed to X-ray film at −80°C for 3 days. The probe was then stripped off from the nitrocellulose paper with boiling water. The paper was then rehybridized with the HTLV-I pMT-2 probe and washed under the same stringency conditions that were used with the pH6neo probe.

DNA sequencing. SM-3 lymphocyte DNA prepared from a 12-week culture and genomic DNAs from HTLV-II cell line 729(pH6neo) and other control cell lines were amplified by HTLV-I and -II generic primers SK43 and SK44 (10) in a PCR hot-start reaction. Ten microliters of PCR product from the first round of amplification was used as the template for the

second-round PCR assay. Ten microliters of double-amplified DNA was used for automated direct DNA sequencing (Applied Biosystems, model 373A, version 1.0.2). The procedures were as recommended by the manufacturer (50). A Bio-spin 30 column (Bio-Rad) was used to separate free dye terminators from the labeled DNA fragments.

IFA. The expression of STLV-II viral antigens in cells cocultured with PBMCs from spider monkeys was determined by an indirect immunofluorescent-antibody assay (IFA). The acetone-fixed cells on microscope slides were incubated with HTLV-II carrier serum (1:200 dilution) or plasma from anti-RP-IIB-seropositive spider monkeys (1:20 dilution) and were exposed to fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin G1 or fluorescein isothiocyanate-conjugated rabbit anti-monkey immunoglobulin G (1:64 dilution; Sigma Chemical Co.). The assay was scored with a fluorescence microscope.

Electron microscopy examination. Fifty million BJAB cells which had been cocultivated with lymphocytes from SM-3 were pelleted and fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer with 8% sucrose, pH 7.4, at room temperature for 60 min. The cells were then washed in the same buffer overnight and postfixed in 1% buffered osmium tetroxide and 1.5% potassium ferricyanide for 1 h at 4°C. After washing in cacodylate buffer for 1 h and dehydration in graded ethanol, the cell pellets were stained in 1% phosphotungstic acid for 30 min in absolute ethanol. The cells were embedded in eponate-12 for morphological study with Zeiss EM-900 and JEM-1200 electron microscopes (56).

Sucrose gradients and RT assay. The viral particles were collected from the culture medium of either SM-3 lymphocyte culture or the HTLV-II cell line 729(pH6neo) through ultracentrifugation. The viral particles were then resuspended in 0.5 ml of phosphate-buffered saline and were loaded onto a 20 to 60% (wt/vol) sucrose gradient solution containing 10 mM Tris-HCl (pH 7.4), 0.1 M NaCl, and 1 mM EDTA, and then they were centrifuged at 18°C for 18 h. Different fractions were collected from the bottom of the gradient, and portions were assayed for reverse transcriptase (RT) activity. For the RT assay, poly(rA) · oligo(dT)$_{12-18}$ (Boehringer Mannheim) was used as the template primer in a standard Mg^{2+} or Mn^{2+} divalent cation-dependent RT assay (42).

Nucleotide sequence accession number. The nucleotide sequence of SM-3 STLV-II has been deposited with the LANL data base under the accession number L25271.

RESULTS

Among the different species of New World monkeys, 5 of 9 (56%) spider monkeys and 1 of 47 (2%) owl monkeys showed antibody reactivity to HTLV-II recombinant envelope glycoprotein RP-IIB by Western blot assay (Table 1). None of the marmosets or the capuchin, howler, or squirrel monkeys that were tested showed antibody reactivity to RP-IIB. In addition, none of the New World monkeys that were tested had antibody reactivity to RP-B1, an HTLV-I-specific envelope recombinant protein. Figure 1 shows the Western blot result of sera from five spider monkeys (SM-1 to SM-5) to RP-IIB and RP-B1. According to the Western blot analysis, SM-2 and her two male offspring, SM-3 and SM-5, may have been infected with a virus related to HTLV-II.

PBMCs from SM-2, SM-3, and SM-5 were collected, stimulated with phytohemagglutinin-L, and cultured in RPMI 1640 medium with interleukin-2. Ten days later, the cells were cocultivated with several human cell lines including a T-cell line (SUP-T1), an Epstein-Barr virus-negative B-cell line (BJAB), and a promonocyte line (THP-1). It has been demonstrated that HTLV-II, but not HTLV-I, can infect and induce syncytium formation in BJAB cells (15, 16). Judged by RT activity, IFA, PCR, and electron microscopy examination, the virus present in the PBMCs from spider monkeys could infect and replicate in both SUP-T1 and BJAB cells but not in THP-1 cells (some of the data are shown below). However, according to microscopic examination, the virus did not cause any cytopathic effect in SUP-T1 and BJAB cells. Several attempts to immortalize human cord blood lymphocytes by cocultivation with the HTLV-II-related virus-infected PBMCs from spider monkeys have been unsuccessful.

To confirm that the STLV infecting spider monkeys was related to HTLV-II, genomic DNAs from the PBMCs of spider monkeys' or from different cocultures were extracted and subjected to gene amplification with different HTLV-II-specific or generic primer pairs by PCR. As illustrated in Fig. 2, dot blots of PCR products amplified by HTLV-II-specific primers SK58 and SK59 or HTLV-I and -II generic primers SK110 and SK111 were hybridized with HTLV-II-specific probes (SK60 and SK188) or an HTLV-I-specific probe (SK112), respectively. In comparison with the control BJAB cells (Fig. 2, lane 3), the BJAB cells cocultivated with PBMCs from monkey SM-2 contained HTLV-II-specific signals, which

TABLE 1. Rates of seropositivity to HTLV-I- or HTLV-II-specific recombinant proteins of different species of New World monkeys (members of the family *Platyrrhini*) by Western blot assays

New World Monkey	No. of serum samples	No. with seropositivity to[a]	
		RP-B1	RP-IIB (%)
Spider monkey (*A. fusciceps*)	9	0	5 (56)
Owl monkey (*A. trivirgatus*)	47	0	1 (2)
Marmoset			
C. jacchus	2	0	0
S. oedipus	12	0	0
S. flaviceps	4	0	0
Capuchin monkey (*C. albifrons*)	10	0	0
Howler monkey (*A. villosa*)	20	0	0
Squirrel monkey (*S. sciureus*)	2	0	0

[a] RP-B1 contains amino acid residues 166 to 201 of the HTLV-I envelope glycoprotein, and RP-IIB contains amino acid residues 96 to 235 of the HTLV-II envelope glycoprotein.

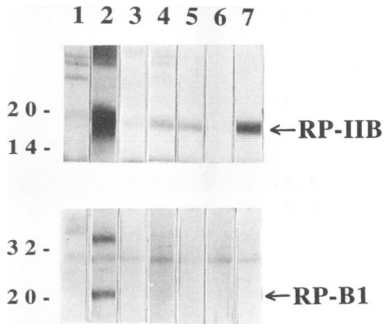

FIG. 1. Antibody reactivities of serum samples from five spider monkeys, SM-1 to SM-5 (lanes 3 to 7, respectively) to HTLV-II envelope recombinant protein (RP-BII) or HTLV-I envelope recombinant protein (RP-B1) by Western blot assay. Lanes: 1, HTLV-I- and -II-negative serum; 2, HTLV-II- or HTLV-I-positive serum for the RP-IIB or RP-B1 assay, respectively. Sizes (in kilodaltons) are at the left.

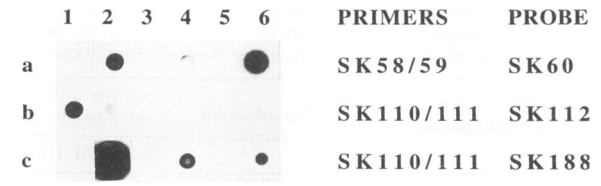

FIG. 2. Dot blot assay of PCR products of lymphocyte DNAs from two spider monkeys. HTLV-II-specific primers (SK58-SK59) or HTLV-I and II generic primers (SK110-SK111) were used for the PCR. HTLV-II-specific probes (SK60 and SK188) or an HTLV-I-specific probe (SK112) was used for the dot blot assay. The template DNAs used were prepared from HTLV-I-positive HUT-102 cells (lane 1), HTLV-II-positive 729(pH6neo) cells (lane 2), BJAB cells (lane 3), lymphocytes from SM-2 cocultivated with BJAB cells (lane 4), Sup-T1 cells (lane 5), or lymphocytes from SM-3 cocultivated with Sup-T1 cells (lane 6).

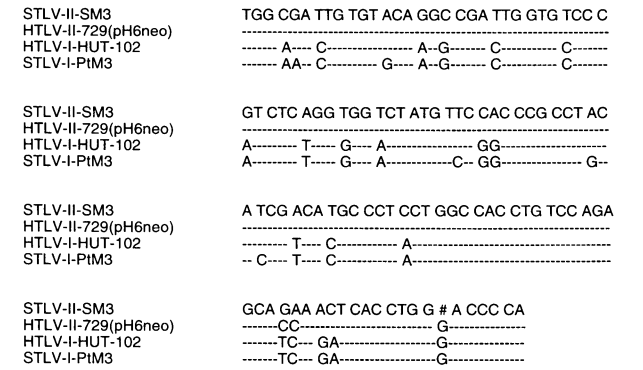

FIG. 4. Partial nucleotide sequence of the *X* region of STLV-II isolated from SM-3; comparison with the *X* regions of HTLV-II 729(pH6neo), HTLV-I HUT-102, and STLV-I PtM3. The sequences shown in the figure are equivalent to that of HTLV-II provirus sequence 7269 to 7383 (41). #, deletion.

were amplified by SK110-SK111 primers and detected with the SK188 probe (lane 4, row c). The HTLV-II-related viral signals were also detected in the coculture of SUP-T1 cells with PBMCs from monkey SM-3 (Fig. 2, lane 6, rows a and c) or in the coculture of BJAB cells and SM-3 PBMCs (data not shown). No HTLV-I-related viral signal was detected in the coculture of BJAB cells with SM-2 PBMCs (lane 4, row b) or the coculture of SUP-T1 cells with SM-3 PBMCs (lane 6, row b) by an HTLV-I-specific probe, SK112.

Southern hybridization analysis demonstrated the presence of the HTLV-II-related provirus in the primary and cultured lymphocyte DNA of SM-3 (Fig. 3). After digestion with different restriction endonucleases, genomic DNA from the coculture of BJAB cells with PBMCs from SM-3 contained fragments which hybridized with the HTLV-II full-length provirus probe pH6neo (4) under relatively high-stringency conditions (Fig. 3, left panel, lanes 5 to 9). In contrast, when an HTLV-I full-length provirus probe, pMT2 (11), was used as a probe, very weak signal could be detected in the HTLV-II-positive cell line 729(pH6neo) (Fig. 3, right panel, lanes 1 and 2) whereas no proviral signals could be detected in the coculture of BJAB cells with SM-3 PBMCs (Fig. 3, right panel, lanes 5 to 9). Furthermore, the restriction endonuclease pattern of the HTLV-II-related provirus (STLV-II) was different from that of HTLV-II; e.g., there was no *Bam*HI site in STLV-II, whereas there were three sites for HTLV-II, and there were two *Hin*dIII-digested subgenomic fragments from STLV-II, whereas this site is missing from HTLV-II.

The open reading frame (ORF) II within the *pX* region (ORF *pX*-II) of HTLV-I HUT-102 cells, HTLV-II 729(pH6neo) cells, and the corresponding region of STLV-II from SM-3 PBMCs or the BJAB coculture were amplified with SK43 and SK44 primers by double PCR and were analyzed by DNA sequencing. As shown in Fig. 4, STLV-II isolated from SM-3 shares 97, 83, and 78% nucleotide homology with HTLV-II, HTLV-I, and STLV-I, respectively. A published

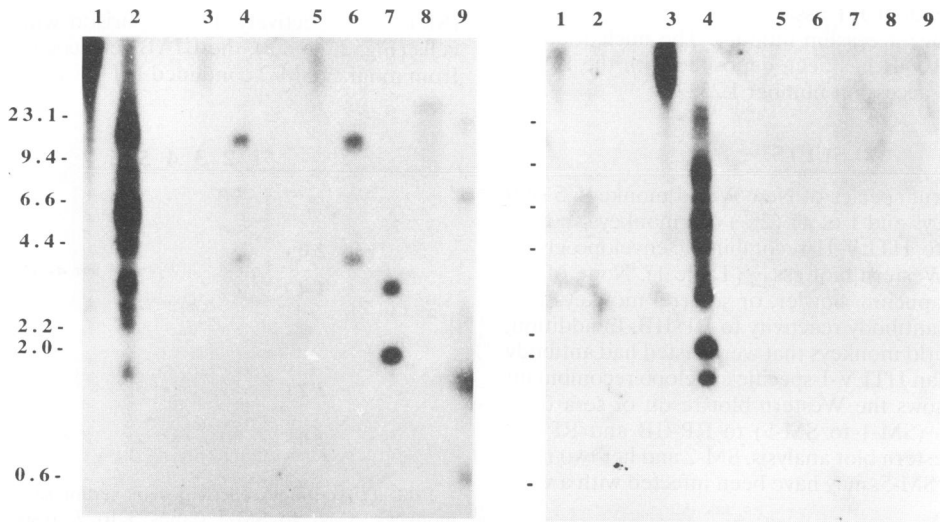

FIG. 3. Southern blot hybridization of an HTLV-II probe, pH6neo (left panel), and an HTLV-I probe, pMT2 (right panel), to restriction enzyme-digested DNAs extracted from BJAB cells cocultivated with lymphocytes from SM-3 (BJAB–SM-3). Lanes for both panels: 1 and 2, undigested or *Pst*I-digested genomic DNAs from HTLV-II-positive 729(pH6neo) cells; lanes 3 and 4, undigested (lane 3) or *Pst*I-digested (lane 4) genomic DNAs from HTLV-I-positive HUT-102 cells; lanes 5 to 9, undigested or *Pst*I-, *Hin*dIII-, *Bam*HI-, or *Eco*RI-digested genomic DNAs from BJAB–SM-3, respectively. Sizes (in kilodaltons) are at the left.

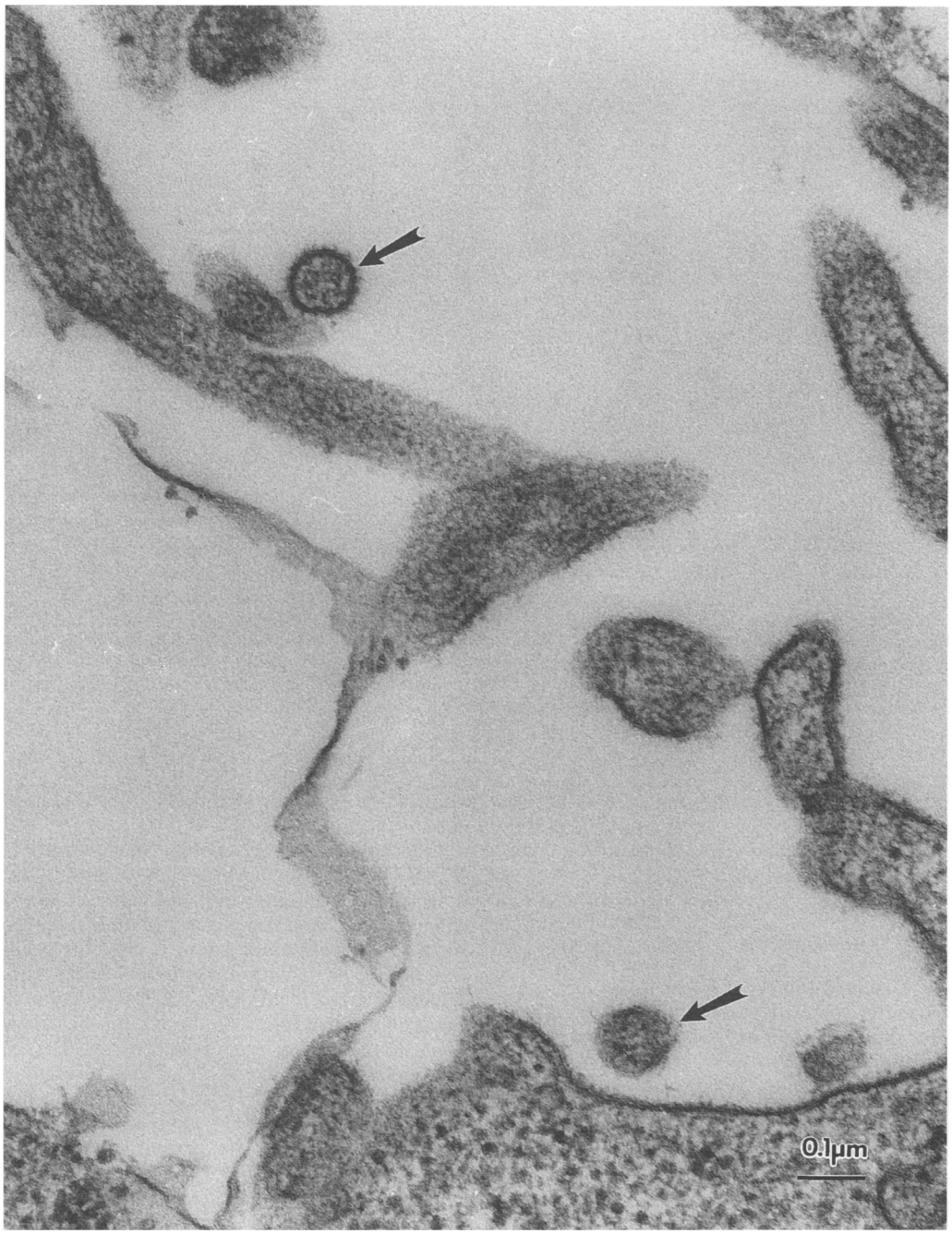

FIG. 5. Electron micrograph of BJAB cells cocultivated with lymphocytes from an STLV-II-infected spider monkey (SM-3). Arrows, viral particles.

sequence of a southeast Asian STLV-I isolate (PtM3) was also included for comparison (58). The sequence data of STLV-II isolated either from fresh SM-3 PBMCs or from the BJAB–SM-3 cocultured cells were identical and have also been confirmed by gene cloning and dideoxynucleotide sequencing. The deduced amino acid sequences of STLV-II Rex or Tax proteins showed 95% homology with the HTLV-II Rex and Tax proteins, respectively.

Electron micrographic studies of SM-3 lymphocytes and the BJAB–SM-3 coculture revealed the presence of type C retrovirus particles that were 100 to 110 nm in diameter and possessed double envelope membranes and a round electron-dense core. As shown in Fig. 5, the STLV-II virions were found outside the infected cell membrane.

STLV-II viral particles with a refractive index of 1.393 (cell density of 1.19 g/cm^3) were harvested from sucrose gradients as indicated by Mn^{2+}- or Mg^{2+}-dependent RT activity. The viral lysate was analyzed with an HTLV-II-positive serum sample by Western blot assay (Fig. 6). In contrast to the control fractions with a refractive index of 1.383 (Fig. 6, lane 1), STLV-II from

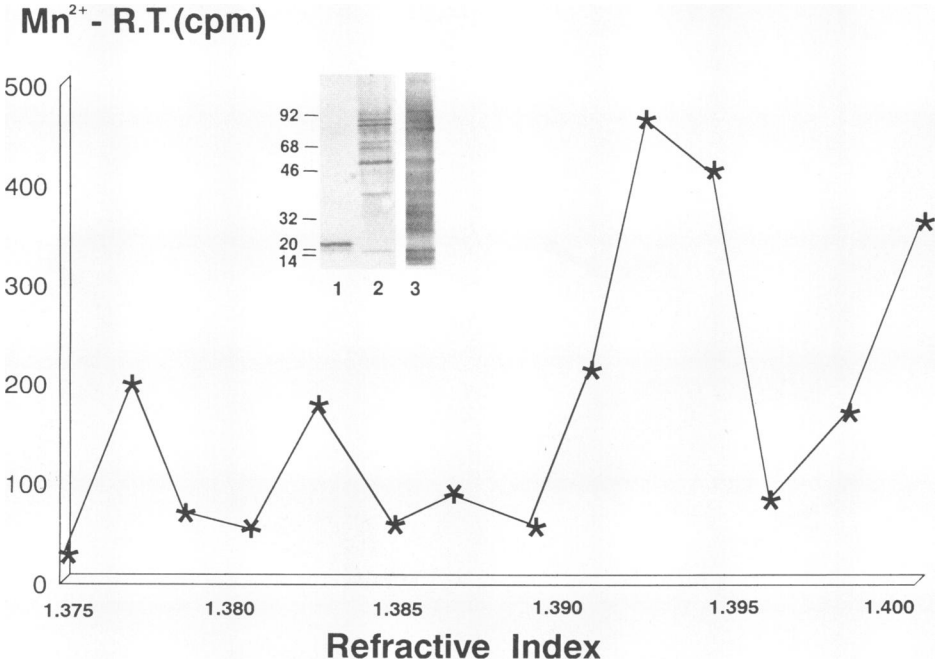

FIG. 6. RT and Western blot assays of different fractions of the sucrose gradients of STLV-II from SM-3. Viral particles with refractive indices of 1.383 and 1.393 (cell density of 1.19 g/cm^3) were harvested from sucrose gradients separately, and their lysates were analyzed with an HTLV-II-positive serum sample by Western blot assays. In contrast to the control lysate with a refractive index of 1.383 (lane 1), STLV-II from SM-3 (lane 2) had an antigen profile similar to that of HTLV-II from the 729(pH6neo) cell line (lane 3).

SM-3 (lane 2) had an antigen profile similar to that of HTLV-II (Fig. 6, lane 3). The STLV-II Gag p55 precursor and p15 proteins were reactive to the HTLV-II-positive human serum.

DISCUSSION

In this study, 5 of 9 spider monkeys and 1 of 47 owl monkeys were found to have serologic evidence of STLV-II infection. Among the 9 spider monkeys used in this study, 8 originated from Panama but were born in two metropolitan zoos in the United States; only 1 spider monkey, which also had antibody reactivity to RP-IIB, had been born in the wild. Recently, Kaplan et al. screened 75 spider monkeys by an HTLV-I enzyme immunoassay (EIA) and found none of them to be seropositive (28). It is possible that the anti-STLV-II antibodies present in the monkey sera were missed by the HTLV-I antigen-based EIA since some HTLV-II-positive serum samples have been missed by such screening tests (5, 21). In addition, the use of anti-human immunoglobulin G in the HTLV-I EIAs may raise concerns regarding the detectability of monkey antibodies in the serum samples. In the present study, instead of using HTLV-I EIAs, we used an HTLV-II recombinant envelope protein, RP-IIB, in a Western blot assay to detect STLV-II infection in New World monkeys. This assay has been proved to be able to detect virtually all HTLV-II carriers, including several intravenous drug users who were seronegative to HTLV-I EIAs (5, 7). In order to cross-react with monkey antibodies, the concentration of biotinylated sheep anti-human immunoglobulin used in the RP-IIB Western blot assay was raised from a dilution of 1/500 to one of 1/100. In addition, fluorescein isothiocyanate-conjugated rabbit anti-monkey immunoglobulin has been used in the IFA to confirm the presence of anti-HTLV antibodies in the monkey sera.

One spider monkey, SM-4, which was not infected with STLV-II, died of cardiomyopathy during the study. SM-4 spleen cells from autopsy had been used to cocultivate with different cell lines for virus isolation. Since multinucleated giant foamy cells were found in SUP-T1 coculture, electron microscopy examination was employed to rule out the presence of simian foamy virus (SFV) infection (9, 23). Typical intracellular particles, 35 to 50 nm in diameter, with an electron-lucent core surrounded by an electron-opaque shell were identified in the SUP-T1 cocultures (data not shown). SFV virus lysates from SM-4 have been used in a Western blot assay to detect anti-SFV antibody in serum samples from SM-1, -2, -3, and -5. The analysis showed that none of them had anti-SFV antibodies (data not shown). In addition, no intracellular viral particles resembling SFV have been found in BJAB cells cocultivated with STLV-II-positive SM-3 PBMCs (Fig. 5).

There are several lines of evidence to suggest that the STLV-II isolate described here is a new simian retrovirus closely related to but distinct from HTLV-II. Morphologically, it is a type C retrovirus with a condensed core that resembles HTLV. Enzymatically, its RT activity is both Mg^{2+} and Mn^{2+} divalent cation dependent. Genetically, it has a high degree of nucleotide homology with HTLV-II, but its restriction endonuclease pattern is distinct from that of HTLV-II. It has been reported that there is 100% sequence homology of the ORF *pX*-II region among different HTLV-II isolates (47). Therefore, the difference in the nucleotide sequence of the ORF *pX*-II region in STLV-II may reflect differences important to the function of its gene products, Tax and Rex. The functional constraints of the gene products of the HTLV-I ORF *pX*

regions have been studied, and there is 96% sequence homology of the ORF *pX*-II region between STLV-I PtM3 and HTLV-I HUT-102 (Fig. 4). The deletion of nucleotide number 7378 in the *X* region of SM-3 STLV-II will affect the ORFs of *tax* and *rex* genes. Further DNA sequencing of the remaining region of the *X* gene may elucidate the functional domains of STLV-II Tax and Rex proteins. In addition, molecular cloning and sequencing of other regions of STLV-II are needed to understand its phylogenetic relationship with other primate T-cell leukemia viruses.

STLV-II can infect and replicate in BJAB cells, but it does not induce syncytium formation upon cocultivation with BJAB cells, in contrast to HTLV-II (15, 16). The syncytium-inducing capability of HTLV-II in BJAB cells has been studied, and its fusogenic domain was found to be located in a 64-amino-acid stretch in gp21 transmembrane protein (39). Sequence analysis of the analogous domain of STLV-II may elucidate the determinants in HTLV-II gp21 that mediate cell fusion.

It has been reported that HTLV-II infection may be endemic in certain New World aboriginal populations which include an Indian tribe, the Guaymis, who live in Panama (41). Since these Indian tribes are relatively isolated and do not have typical risk factors for HTLV-II infection, the endemic HTLV infection may have arisen from close contact with primates, as some have hypothesized regarding the origin of HTLV-I infection (25). Recently, Goubau et al. reported that pygmies from Zaire have antibody reactivities to an HTLV-II-specific recombinant protein, K55 (14). The confirmation of HTLV-II in these populations requires further investigation; however, more studies of STLV-II infection in Old World primates appear necessary.

Since three of the five STLV-II-seropositive monkeys belonged to a family grouping, it seems likely that STLV-II infection can be transmitted perinatally, like STLV-I and the HTLVs. Further serological studies of spider monkeys and other New World primate species from the wild are needed. Our data suggest that New World primates may be infected with STLV-II, whereas Old World primates and apes are naturally infected with STLV-I (17, 59). Further studies of STLV-II may help us understand this curious phylogenic distribution. New fossil evidence suggests that the time of origin of simian primates may be pushed back into the Paleocene period, which means that direct migration of simians between Africa and South America is more likely (33). If STLV-I and STLV-II are ancient primate retroviruses, they may be useful in studying aspects of primate evolution, such as the divergence of New World primates. The low genetic drift and limited horizontal transmission of the human counterparts, HTLV-I and HTLV-II, suggests the utility of STLVs for these types of investigation (13).

Finally, since no disease is definitively associated with HTLV-II infection, spider monkeys infected with STLV-II may serve as a useful animal model to study the pathogenicity of HTLV-II infection.

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