Human T Cell Leukemia Virus Type 1 Up-Regulation after Simian Immunodeficiency Virus–1 Coinfection in the Nonhuman Primate

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The effects that human T cell leukemia virus (HTLV) type 1 and simian immunodeficiency virus (SIV) coinfection have on HTLV-1 dynamics and disease progression were tested in a nonhuman primate model. Seven rhesus macaques were experimentally inoculated with HTLV-1, and a persistent infection was established. Coinfection with SIV/smB670 resulted in increased HTLV-1 p19 antigens in peripheral blood mononuclear cells and HTLV-1 proviral loads. Circulating CD2+ and CD8+ T lymphocytes increased over preinoculation levels, along with a progressive decrease in CD4+ T cells, typical for terminal SIV disease. Finally documented was the striking emergence of up to 19% of HTLV-associated “flower cell” lymphocytes in the circulation, as seen in patients with adult T cell leukemia/lymphoma. CD8+CD25+ T cell subpopulation increases were positively correlated with flower cell appearance and suggested their possible role in this process. We conclude that SIV may have the potential to up-regulate HTLV-1 and disease expression.

Human T cell leukemia virus (HTLV) type 1 is an important pathogen worldwide known to cause a life-long chronic infection that may lead to adult T cell leukemia/lymphoma (ATLL) and a neurodegenerative disease known as tropical spastic paraparesis/HTLV-1–associated myelopathy (TSP/HAM) [1–5]. In certain geographic regions, HTLV-1 coinfection in HIV–positive individuals is high (5%–10%) [6, 7], and recent data suggest that HIV/HTLV-1 coinfection is associated with an increase in HTLV-1 expression and disease [8–10]. The effects of highly active antiretroviral therapy (HAART) in control of HTLV-1 replication is unclear [11], and concern exists for the late emergence of HTLV-1 disease manifestations given that dually infected persons live longer in the era of HAART because their HIV infection is controlled. This is evidenced by high observed rates of TSP/HAM in patient population studies of HIV/HTLV coinfection [12].

Simian T cell leukemia virus (STLV)–1, the simian counterpart of HTLV-1, naturally infects Old World monkeys and shares virologic, immunological, molecular, and pathological features with HTLV-1 [13–15]. Phylogenetic relationships of most virus subtypes indicate that STLV-1 is the simian ancestor of HTLV-1 that resulted from cross-species transmission from multiple nonhuman primates (NHP) to humans [16–20]. In naturally infected monkeys, STLV-1 pathogenesis is similar to HTLV-1 in humans, causing ATLL-like pathological features in a minority of individuals after a long period of latency [21–24].
HTLV-1 infection was demonstrated in several monkey species inoculated with MT-2 cells, Ra-1 cells, or autologous HTLV-1-infected cell lines [25, 26]. Rhesus macaques and squirrel monkeys have been successfully infected with HTLV-1 and used as NHP models for studying HTLV-1 pathogenesis, drug testing, and vaccine trials [27–32].

The current study employed the rhesus model to test the hypothesis that HIV-HTLV-1 coinfection is associated with increased HTLV-1 expression and disease. Rhesus macaques were infected with a primary clinical isolate of HTLV-1 from an individual with associated clinical diseases, TSP/HAM and polymyositis [27]. These macaques were then either simultaneously or serially coinfected with a pathogenic simian immunodeficiency virus (SIV) [33, 34]. Macaques were followed longitudinally for HTLV-1 by culture, polymerase chain reaction (PCR), serologic, and flow cytometry testing to assess virological and immunological responses and for hematological parameters to assess ATLL cell emergence in the circulation.

**MATERIALS AND METHODS**

**Macaques.** Seven Chinese rhesus macaques (Macaca mulatta; 6 males and 1 female) were used. Macaques were 1–2 years old and confirmed seronegative and PCR negative for STLV-1 and SIV at the study initiation. Three Indian and 3 Chinese rhesus macaques from earlier studies served as SIV–monoinfected controls (L.N.M., unpublished data) [35]. Naturally STLV-1–infected macaques were also utilized as controls for baseline values.

All animal housing, care, and research were performed in conformance with the Guide for the Care and Use of Laboratory Animals (National Research Council) and the guidelines at the Tulane National Primate Research Center, were fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and were in accordance with Animal Welfare Act guidelines. Protocols were approved by the Institutional Animal Care and Use Committee. Physical exams were performed either biweekly or monthly, and macaques were provided full supportive care. Moribund macaques were humanely killed, and necropsies were performed.

**Viruses.** A CD4+ HTLV-1–producing cell line, denoted “HTLV$_{en}$,” was used for inoculation preparation. HTLV$_{en}$ was established from cultured peripheral blood mononuclear cells (PBMCs) of a patient with TSP/HAM, polymyositis, uveitis, and lymphoid interstitial pneumonitis [36]. The inoculum was shown to be free of HIV and Mycoplasma by culture and PCR. Macaques were inoculated intravenously (iv) once or twice with $\sim 1 \times 10^7$ phytomagglutinin-stimulated autologous PBMCs co-cultured with $5 \times 10^6$ irradiated HTLV$_{en}$-cells, as described elsewhere [27] (figure 1). After 1–2 years of HTLV-1 infection, subjects P574, P671, M744, P442, and P111 were iv inoculated with 50–100 TCID$_{50}$ of freshly thawed, cell-free, cryopreserved stock of SIVsmB670. Macaque N365 was simultaneously iv inoculated with both PBMC/HTLV$_{en}$ coculture and cell-free SIVsmB670. An additional control, rhesus N401, was mock inoculated with autologous PBMCs and later inoculated with the HTLV$_{en}$–PBMC coculture, as described above.

**Clinical testing, viral detection, and serologic analysis.** Routine monthly clinical testing included complete blood counts, differential white blood cell (WBC) counts, chemistry levels, and parasite stool examination. Additional blood was collected for virologic, serologic, and flow cytometric evaluations. PBMCs were isolated from blood by density Ficoll-Hypaque separation, for both culture and cryopreservation for storage and later nucleic acid extraction and PCR.

HTLV-1 p19 antigen detection in PBMC culture, serologic analysis, genomic DNA isolation, and end-point PCR of circulating PBMC genomic DNA were performed as described elsewhere [27]. Culture supernatants were harvested at days 7, 14, and 21 for HTLV-1 viral p19 antigen detection, by use of a commercial kit (Zeptometrix). A positive culture was defined as antigen detection at any of the times points tested. Serologic assays were performed using a commercial ELISA kit (Abbott Laboratories). Serial 2-fold dilutions of serum (1:20–1:1280) were assayed in accordance with the manufacturer’s instructions. A spectrophotometric reading of $\geq 2.5$ times the optical density reading of a serum sample from an uninfected control macaque was obtained as a positive titer.

Plasma samples for SIV RNA viral antigen analysis were cryopreserved at $–70^\circ$C until analysis. SIV antigenemia was determined by SIV p27 core antigen ELISA (Beckman Coulter) in accordance with the manufacturer’s instructions.

**Virus load quantification.** Real-time PCR quantification was performed with the Prism 7700 sequence detection system (Applied Biosystems ABI) for HTLV-1tax and RNase P amplification. Primers were as follows: HTLV tax, forward primer 7930F, 5′-GCC CTA ATA ATT CCA CCC GAA GAC T-3′; reverse primer 8040R, 5′-GTT TGA GTG CAG ACA CTG CTG-3′; and probe 7991P, 5′-Reporter dye-FAM-CCG TCA CGC TAA CAG -TAMRA-Quencher. Primers were selected according to parameters defined by the Primer Express software (version 2.0; ABI) and were based on strain J02029 [37]. Plasmid pM72 containing the full HTLV-1 tax/rex coding region in pUC13 was used as HTLV-1 control DNA to generate the standard curve [38]. Fifty-microliter reactions contained 1× $10^{-1}$–1× $10^4$ copies of viral control DNA or 500 ng of genomic PBMC DNA and the following: 1× TaqMan buffer A; 200 $\mu$mol/L each dATP, dCTP, and dGTP; 400 $\mu$mol/L dUTP; 3.5 mmol/L MgCl$_2$; 300 $\mu$mol/L HTLV primers; 200 $\mu$mol/L HTLV-1 probe; 0.625 U of AmpliTaq Gold; and 0.25 U of uracil N-glycosylase. Reactions were amplified in duplicate in 96-well optical-grade PCR plates. The reaction proceeded as follows: 2
Figure 1. Macaque culture and serologic results. Results are summarized for the experimentally inoculated macaques, N401, P574, N365, P671, M744, and P442 (P111 data not shown). Each virus inoculum is shown with the virus name boxed and positioned above the inoculation date, which is shown in months after initial human T cell leukemia virus (HTLV) type 1 inoculation. Inoculation with HTLV-1 is shown for M744, P574, P671, and P442. These same macaques were later inoculated with the second virus, simian immunodeficiency virus (SIV). One macaque (N365) was simultaneously inoculated with HTLV-1 and SIV. N401, originally the uninfected control, received a mock infection initially. After 20 months, N401 was inoculated with HTLV-1 and used as the single HTLV-1–infected control for the coinfection experiments. Antibody response to HTLV-1 was tested by EIA, and resultant titers are shown longitudinally by columns. The presence of HTLV-1 p19 in peripheral blood mononuclear cell culture is shown as + or − below each of the monthly culture points.

min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C and 60 s at 60°C. Viral copy numbers were interpolated from the plasmid control regression curve. Finally, RNase P, a single-copy gene (2 copies/diploid cell) was used as an endogenous DNA reference to accurately quantify cell equivalents and normalize sample variability. RNase P primers, probe, and standards were commercially obtained (ABI). Final HTLV-1 proviral copy number was calculated for each sample as follows: HTLV-1 copies/[RNase P copies/2] × (1 × 10⁶ cells). The lower limit of the assay was 1 copy/1 × 10⁶ cells. Samples with undetectable virus were scored as 10 copies/1 × 10⁶ cells.

Flow cytometry and quantification of T lymphocyte populations. EDTA-anticoagulated whole blood samples were evaluated for lymphocyte subsets by fluorochrome-conjugated
Figure 2. Human T cell leukemia virus (HTLV) type 1 provirus loads after simian immunodeficiency virus (SIV) inoculation. Real-time polymerase chain reaction was used to quantify virus loads, HTLV-1 provirus copies found in the peripheral blood mononuclear cells of dually HTLV/SIV-infected macaques (see Materials and Methods). Copies of HTLV-1 provirus for each macaque on each of the months after SIV inoculation are shown as copies/1 × 10^6 cells. The lower limit of the assay was 1 copy/1 × 10^6 cells. Samples with undetectable virus loads were scored as 10 copies/1 × 10^6 cells.

monoclonal antibody staining and flow cytometry, as described elsewhere [34]. CD2+ T cells and CD20+ B cells were identified with anti-T11 and anti-B1 (Beckman Coulter). CD4+ T cells, CD8+ T cells, and CD25+ T cells were identified with anti-CD4 (clone L200), anti-CD8 (clone RPA T8), and anti-CD25 (clone 2A3) (BD Biosciences). Erythrocytes were lysed, and leukocytes were fixed with ImmunoPrep reagents using the TQ-Prep System (Beckman Coulter). Data acquisition and analysis were performed on a FACSCalibur device using Cell Quest software (BD Biosciences). Absolute numbers of cells were determined by calculating the percentage of the subset multiplied by total lymphocytes.

Enumeration of “flower cells.” Giemsa-stained peripheral blood smears were prepared, 100 WBCs were counted, lineage was assessed, and smears were analyzed for the presence of the abnormally lobulated flower cell lymphocytes of HTLV infection [39]. Mean numbers of flower cells in the HTLV/SIV group compared with those in the controls were analyzed using an independent t test with unequal variances assumed to 3 months. Multiple comparisons required P values to be adjusted using the Bonferroni correction factor.

To control for spontaneous occurrence of flower cells in uninfected or single virus–infected NHP and to establish baselines, blood smear slides from previously tested SIV and STLV-1–negative or singly STLV-1–positive colony macaques were evaluated. As a control for accuracy, an independent investigator reading and quantification of flower cells was performed on a blinded sample set including smears from both virus-positive and -negative macaques. Readings had a high correlation (96%) between investigators.

RESULTS

Rhesus macaques were inoculated with HTLV-1K1–infected cells and SIVsmB670 to study the virological, immunological, and clinical effects of dual retroviral infection. Five macaques (P671, P574, P442, P111, and N401) were initially singly inoculated with HTLV-1 (figure 1). Two macaques from a previous study were also utilized: M744, inoculated with HTLV-1 only; and N365, inoculated with HTLV-1 simultaneously with SIV [27]. Five persistently HTLV-1–infected macaques (M744, P574, P671, P111, and P442) were later inoculated with 50–100 TCID50 of SIVsmB670 and monitored along with N365, the other dually HTLV-1/SIV–infected macaque. SIV infection was confirmed in all coinfected macaques by positive SIV p27 antigen in the circulation (data not shown). Macaque N401 remained as a singly HTLV-1–infected control.

Progression to AIDS was observed at 3–13 months in 5 of 6 the serially coinfected macaques and at 28 months in the simultaneously coinfected macaque, N365. These macaques died of colitis, cachexia, dyspnea, lymphoid hyperplasia, and opportunistic infections, typical for terminal immunodeficiency.
Figure 3. Lymphocyte subset populations after simian immunodeficiency virus (SIV) inoculation. Results are shown for CD2+ T lymphocytes (A), CD8+ T lymphocytes (B), and CD4+ T lymphocytes (C), for the dually human T cell leukemia virus (HTLV) type 1/SIV–infected macaques and the 1 HTLV-1–infected control macaque at each monthly time point after SIV inoculation. Resultant cell nos. are shown as absolute nos. of cells/mm³.

Macaque P442 did not show clinical signs of AIDS. For this macaque, however, an elected necropsy 5 months after SIV coinfection showed severe lymphoid hyperplasia in both lymph nodes and spleen.

After HTLV-1 inoculation, longitudinal samples for all 7 macaques confirmed HTLV-1 infection by antigen detection in PBMC culture, serologic analysis, and end-point PCR. Figure 1 depicts these culture and serologic results for 6 of the 7 macaques (excluding P111). HTLV p19 antigens were detected in PBMC cultures for 6 of the inoculated macaques. HTLV-p19 antigen in cultures were positive before or during peak antibody production with differences in peak responses cor-
related with viral clearance. Strong peak antibody responses (1:1280) were shown in M744 and P442; moderate responses (1:320–1:640) in N365 and P671; and low responses (1:40–1:20) in P574 and N401. P111 with intermittent positive culture and PCR results never mounted a detectable antibody response (data not shown). Macaque P442, with high antibody response, was culture negative for >2 years. N401 antibody decrease was associated with intermittent positive cultures. P574, P671, M744, and P442 cultures decreased to undetectable levels, despite persistence of infection as documented by PCR (data not shown). After SIV inoculation, HTLV-1 antibody levels were reduced further and were correlated with positive HTLV p19 detection in cultures in all coinfected macaques.

After SIV coinfection, HTLV loads were monitored and quantified by real-time PCR amplification of the HTLV-1 tax gene. At baseline, HTLV-1 tax was either undetectable (M744, P574, P442, and N365) or low (P671 and P111), with only 37.8 and 36.3 provirus copies/10^6 PBMCs (figure 2). After SIVsmB670 inoculation, distinct increases were documented in 4 macaques by 2 weeks, progressing to peak levels over 1 to several months. Peak levels of HTLV provirus were as follows: P111, 83 copies/10^6 PBMCs (1 month); P574, 367 copies/10^6 PBMCs (1.5 months); P671, 156 copies/10^6 PBMCs (2 months); and M744, 68 copies/10^6 PBMCs (2 months). N365, simultaneously HTLV-1/SIV coinfected, was more delayed, with provirus emerging at 4 months after infection and progressing to a peak of 164 copies/10^6 PBMCs at 5 months. Macaque P442 remained completely negative by PCR for HTLV-1K7 sequences in PBMCs throughout the entire time of monitoring.

T cell subset population changes after SIV infection were evaluated by flow cytometry. Three Indian and 3 Chinese rhesus from earlier studies were used as historical controls for singly

**Figure 4.** Comparison of CD25^+CD4^+ and CD25^+CD8^+ T lymphocytes with presence of flower cells for the macaques P574, P671, P111, and P442, at each monthly time point after simian immunodeficiency virus (SIV) inoculation. Flow cytometry results are shown as absolute no. of cells/mm^3 and are shown on the left Y-axis. Flower cells are shown as nos./100 white blood cells from the differential analysis of the peripheral blood smear and are shown on the right Y-axis.

**Figure 5.** Flower cells in a peripheral blood smear from a macaque experimentally inoculated with human T cell leukemia virus type 1. Two multilobulated flower cells are shown in the larger field, and a single flower cell is shown enlarged in the inset.
SIV-infected macaques. These SIV-positive macaques showed
CD2+ T lymphocytes ranging from 1000 to 3100 cells/mm3, and
CD8+ T lymphocytes decreasing to only a few hundred cells
per cubic millimeter within 1 year (L.N.M., unpublished data)
[34]. No sharp increases in either CD2+ or CD8+ T cell pop-
ulations were noted after SIV monoinfection.

Baseline CD2+ T lymphocytes in the study macaques ranged
from 1230 to 3262 cells/mm3 (mean, 2433 cells/mm3) (figure
3A). After SIV infection, at 0.5, 1, and 2.5 months, values in
coinfected macaques (M744, P671, P442, and N365) increased
200%–290% over baseline (3479–6790 cells/mm3) and above
the singly HTLV-1–infected control (522–2159 cells/mm3).
CD8+ T lymphocytes increased 190%–330% in 5 coinfect-
ed macaques (M744, P671, P111, and P442) and above
baseline values (86–104 cells/mm3) paralleled total CD2+ cells and
showed progressive decreases to levels <30 cells/mm3. Macaques
P574 and P111 showed intermittent increases at 2–3 months
after infection but decreased similarly to levels <30 cells/mm3
by 8 months. The smaller CD8+CD25+ subpopulation showed
distinct increases from 0.5 to 2.0 months after SIV infection.

Of particular importance was the abnormal appearance of
multilobulated lymphocytes, resembling the flower cells (figure
5) of ATLL. Flower cells are a hallmark and characteristic find-
ing in HTLV-1 and STLV-1 infections that have progressed to
disease [13, 39, 41]. Readings of archived blood slides from
uninfected or single STLV-1- or SIV-positive macaques estab-
lished baseline values. Those baseline results showed flower cells
present at low levels in the absence of STLV-1: 0%–2% flower
cells (average, 0.8%) in SIV/STLV-uninfected macaques (n =
24); 0%–3% flower cells (average, 0.8%) in SIV-positive/STLV-
negative macaques (n = 23); and 0%–6% flower cells (average,
2.2%) in SIV/STLV-positive macaques (n = 16). The flower
cells observed in the study HTLV-1–positive control, N401,
ranged from 0% to 3% and averaged 1.3%. The coinfect-
ed macaques—M744, P574, P671, P111, and P442—showed dra-
matic increases in flower cells to 7%, 8%, 10%, 6%, and 12%
during the first 3 months (figure 4) (M744 not shown). N365,
the macaque concurrently inoculated with HTLV-1 and SIV,
showed varying levels of flower cells (0%–6%) during the first
2 years after infection, which increased to 19% in a period of
5 months before progression to AIDS and death the following
month (data not shown). Figure 6 shows the calculated
means of flower cells for the coinfect ed macaques compared
with the control for 10 months after SIV inoculation. t test
analysis of flower cells in HTLV-1/SIV–coinfected macaques
(n = 6) through 3 months after infection were shown to be
significantly higher (P = .001) than those observed in the
HTLV-1–positive control macaque (n = 1) and indicate sta-
tistical significance, compared with the Bonferroni-adjusted
critical value of P = .007.

Figure 6. Mean levels of abnormal flower cells in the circulation of the
6 dually infected macaques, compared with that in the human T cell leukemia
virus (HTLV) type 1–positive control macaque, over time after simian immu-
nodeficiency virus (SIV) infection. Because of each macaque’s individual
progression to terminal disease and death, the no. of the group changed:
6 at baseline through 2 months; 5 at 2.5–5 months; 4 at 6 and 7 months;
and 3 at 8 and 10 months. Flower cells are indicated as percent of the
100 white blood cells identified in the differential analysis of the peripheral
blood smear. Values are means for the experimental HTLV/SIV group and
actual values for the control. Actual flower cell percentage for 4 of the
experimental macaques (P574, P671, P111, and P442) are shown in
figure 4.
DISCUSSION

This study showed that SIV coinfection of HTLV-1–infected rhesus monkeys up-regulated HTLV-1 expression and emergence of flower cells in the peripheral circulation. Although the small numbers of macaques in the study precludes powerful statistical analysis, clear changes were documented. After SIV coinfection, specific increases in HTLV-1 p19, HTLV proviral loads, circulating CD2+CD8+ T cells, and abnormal flower cells were clearly evident. The increased occurrence of ≥3% of flower cells in the circulation of coinfected macaques was significantly higher than in the control, yet the macaques maintained normal WBC counts. These findings are consistent with the pattern seen in chronic and smoldering forms of adult T cell leukemia/lymphoma [39, 42]. Furthermore, peripheral blood smears of healthy carriers of HTLV-1 rarely if ever show >1% flower cells [43]. The emergence of flower cells in all 6 coinfected macaques during infection also suggests the possibility that HTLV and SIV may increase the risk for development of leukemia and lymphoma in dually infected individuals.

It has been reported that HIV appears to increase the risk for HTLV-1–associated disease, including hematologic malignancies and TSP/HAM [8, 10, 44]. The dynamics of HIV and HTLV coinfections remain unclear, but Beilke et al. [9] showed that coinfections are associated with increased HTLV-1 mRNA in PBMCs. HIV/HTLV coinfections pose diagnostic and prognostic problems for physicians who do not routinely test their HIV-infected patients for HTLV-1 or HTLV-II antibodies.

HIV/HTLV-1 coinfections are often associated with sustained periods of normal or even elevated CD4+ T cell counts [45] and early neurologic complications in the face of normal CD4+ T cell counts [12]. CD4+/CD25+ T cells, specifically the T regulatory (Treg) cell subpopulation, are suggested as the major reservoir for HTLV-1, express virus, down-regulate the normal suppressor function, allow expansion of HTLV-1 tax–specific CD8+ T cells, and contribute to viral pathogenesis [46, 47]. In addition, CD8+CD25+ T cells have been shown to be a viral reservoir in vivo and involved in pathogenesis of HTLV-1–mediated disorders [46–48].

This study clearly supports clinical observations in humans that HIV/HTLV-1 coinfection may up-regulate cell proliferation, HTLV-1 expression, and increase disease potential. After SIV coinfection, CD4+CD25+ cells progressively decreased, with only 2 of 4 macaques showing intermittent and transient increases over the course of terminal decrease. SIV-specific CD4+ Treg cell depletion is a possible cause for this sharp decrease in CD4+CD25+ T cells [49]. Elimination of the Treg cell suppressor function by SIV and resultant immune hyperactivation [49] along with HTLV-1–induced spontaneous lymphoproliferation may also explain the subsequent increases in the CD8+CD25+ T cell subpopulation and the increase in HTLV replication that were documented in the macaques [46, 47]. The emergence of large numbers of flower cells in macaques, resembling those seen in the circulation of patients with ATLL, is particularly striking. The flower cells directly correlated with CD8+/CD25+ T cells and suggested the possible involvement of this subpopulation in disease progression. Additional studies are ongoing to assess this correlation.

Fultz et al. [50] obtained similar results in pig-tailed macaques inoculated with SIV-PBj and STLV-I. During 2 years of follow-up of singly and dually infected macaques, no differences in SIV burdens, onset of disease, or survival were detected. However, in the first coinfected macaque that died of AIDS (1 year after infection), >50% of CD4+ and CD8+ T lymphocytes expressed CD25, and lymph nodes showed a monomorphic population of lymphoblastoid cells. The authors suggested a preleukemic condition due to STLV/SIV coinfection and speculated that SIV may have potentiated STLV-related disease.

The exact mechanism of up-regulated HTLV-1 expression after SIV coinfection cannot be determined through the current studies. However, the possibility exists that flower cells would continue to increase along with T lymphocyte population decrease after SIV infection. This would suggest that loss of immune control, as opposed to immune stimulation, would be a possible mechanism to explain up-regulation of HTLV-1. This is further evidenced by the late increase in HTLV-1 p19 viral antigen detection in PBMC cultures after a decrease in HTLV-1 antibody titers.

Taken together, our results suggest that the SIV/HTLV-1 model of coinfection may be useful for further studies of HTLV-1 leukemogenesis. The model may also prove very useful in testing the efficacy of antiretroviral compounds and antineoplastic agents for the treatment of HTLV-1 disease manifestations.

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