Transformation to continuous growth of primary human T lymphocytes by human T-cell leukemia virus type I X-region genes transduced by a *Herpesvirus saimiri* vector

(thymocytes/cord blood lymphocytes/*tax*/*rex*/lymphoproliferation)

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ABSTRACT   The role of the X region of the genome of the human T-cell leukemia virus type I (HTLV-I) in the immortalization of lymphocytes has been difficult to distinguish from its role in viral replication as this region encodes at least two genes, *tax* and *rex*, required for replication and the expression of viral proteins. To determine whether the X region does encode immortalizing functions, a fragment of the HTLV-I provirus capable of expressing known X-region proteins was inserted into the genome of a transformation-defective, replication-competent *Herpesvirus saimiri*. Infection of fresh mitogen-activated human cord blood and thymocytes yielded immortal T-cell lines that had the same phenotype (CD4+, CD5+, HLA class II+, interleukin 2 receptor α-chain +) as lymphocytes transformed by cocultivation with HTLV-I. These experiments demonstrate that the X region encodes the functions of HTLV-I that immortalize a distinct subpopulation of human T cells. The experiments also demonstrate the utility of the *H. saimiri* vector for the transduction of heterologous genes into human T cells.

The human T-cell leukemia virus type I (HTLV-I) (1), a retrovirus, is the etiological agent of adult T-cell leukemia-lymphoma (ATLL), an aggressive neoplasm of CD4+ T cells. Tumor cell lines derived from ATLL patients retain many of the characteristics of the tumor cells themselves, including detectable levels of surface CD4 and interleukin 2 (IL-2) receptor (2, 3). Typically, ATLL-derived cell lines also produce HTLV-I virus particles (4). Fresh human T cells may be altered to permanent growth by cocultivation with the virus-producing ATLL cell lines, and the T cells so derived closely resemble tumor cell lines established from patients (5–7). These observations suggest that HTLV-I possesses an immortalizing genetic function. Molecular and biochemical studies reveal that in addition to the genes common to all retroviruses encoding virion structural proteins and replicative enzymes, the genome of HTLV-I contains an additional segment of 1600 nucleotides located near the 3' end called the X region (8). It has been proposed that the X region encodes the immortalizing functions of the virus. However, it has been difficult to distinguish immortalization from replication functions as the X region encodes at least two genes, the trans-activator gene, *tax* (9, 10), and the regulator of expression of virion proteins, *rex* (11, 12), that are required for virus replication.

To determine whether the X region encodes the gene products that induce immortalization as well as replication functions, a segment of a HTLV-I provirus capable of expression of known X-region functions was inserted into the genome of a transformation-defective, replication-competent

Herpesvirus saimiri. Infection of fresh, mitogen-activated human cord blood lymphocytes and thymocytes with the recombinant viruses yielded continuous T-cell lines of the same phenotype (CD4+, CD5+, CD8−, HLA class II+, IL-2 receptor α-chain +) as cells cocultivated with HTLV-I-infected cells. These experiments demonstrate that the X region encodes the functions of HTLV-I that immortalize a distinct subpopulation of human T cells.

MATERIALS AND METHODS

Preparation of Recombinant *H. saimiri*. *H. saimiri* and viral DNA were prepared as described (13, 14). To obtain recombinant viruses, the plasmids pSIneo and pRÜ neo were cut with *Kpn* I and *Eco*RI, respectively, and added to genomic DNA of *H. saimiri* strain 11S4. Plasmid (2–4 μg) and viral DNAs (0.2–0.4 μg) were cotransfected into OMK cells using the calcium phosphate method (15). Two viral stocks (I and II) were obtained from cotransfection with prÜneoHI (I) and pSIneoHI (II). Selection and purification of recombinants will be described elsewhere (37). Briefly, virus from both stocks was passaged three times on G418-treated OMK cells. Single viral plaques were isolated, expanded, and assayed for the presence of heterologous sequences by dot spot hybridization, using ³²P-labeled pHISL-ESX DNA. Viral stocks resulting from plaques II-1, II-40, III-21, III-22, and III-38 were analyzed in closer detail.

Characterization of Recombinant Viruses. For Southern blot analysis, viral and cellular DNA was extracted as described (16, 37). Twenty to 50 ng of recombinant *H. saimiri* DNA or 2–5 μg of cellular DNA was digested with restriction enzymes, separated on 0.8% agarose gels, and blotted onto nitrocellulose filters. For the detection of high molecular weight superhelical DNA, transformed lymphocytes were carefully lysed on the top of a vertical slab gel following the protocol of Gardella *et al.* (17). Cellular DNA was separated electrophoretically into chromosomal, episomal, and linear-degraded fractions. To estimate the copy number of viral episomes, gels were loaded with 10^6 persistently infected lymphocytes and, for reference, with increasing amounts of cells of the tumor cell line 1670. This cell line has been shown to contain about 210 genome equivalents of viral DNA (18). To visualize the recombinant viral sequences, Gardella-type gels were blotted and hybridized to X-region DNA, neoʳ-gene DNA, or *H. saimiri Kpn* I-D fragment (19). DNA·DNA hybridizations were performed following standard protocols

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at 20–25°C below melting temperature in the presence of 50% formamide.

Isolation and Infection of Primary Human Lymphocytes. Human primary lymphocytes were prepared from heparin-treated cord blood by Ficoll/Hypaque (Sigma) density gradient centrifugation and stimulated for 48 hr with 10 μg of phytohemagglutinin (PHA) per ml. Human thymocytes were isolated from a pediatric patient and stimulated with 10 μg of PHA per ml and 10 ng of phorbol 12-myristate 13-acetate (PMA) per ml for 24 hr. Cord blood cells from donor A were depleted of CD8$^+$ cells by immunomagnetic cell sorting (Dynal, Oslo). Cord blood cells (3×10^6) from three different donors (Karl, Kati, A) and thymocytes (10×10^6) were infected with 1.5 ml of different recombinant viral stocks 3–5 days after stimulation. Infected lymphocytes were kept in RPMI 1640 medium supplemented with 20% fetal calf serum and 20 units of IL-2 per ml. The cell cultures were split 1:2 once a week beginning at 3 weeks after infection.

The human thymocytes were cocultivated with HTLV-I-producing cell lines at the same time they were infected with *H. saimiri* recombinants. PHA- and PMA-stimulated thymocytes were cocultivated with HUT 102 or C91PL cells that had been treated with 100 μg of mitomycin C per ml for 1 hr at 37°C. The HTLV-I-infected cells were washed extensively and mixed with thymocytes at a 1:4 ratio. The cells were centrifuged together, gently resuspended to maintain clumping, and cultured in IL-2-containing RPMI medium with 20% fetal calf serum. The cells were split every 2–4 days depending on cell density. Mitomycin C-treated control cultures contained no live cells 3 weeks after cocultivation. Stimulated but uninfected thymocyte controls cultured in IL-2-containing medium had no live cells 8 weeks after cocultivation.

Immunofluorescence and Fluorescence-Activated Cell Sorter Analysis. Monoclonal antibodies against human CD4 (OKT4), CD8 (OKT8), class II (DR) antigens (Ortho Diagnostics), IL-2 receptor α-chain (T-Cell Sciences), and CD5 (Sigma) were used to detect these lymphocyte cell surface markers. Antibody was added and the cells were placed on ice for 30 min. The cells were washed and fluorescein-conjugated goat anti-mouse immunoglobulin (Ortho Diagnostics) was allowed to bind on ice for 30 min. The cells were washed and fixed in 2% formaldehyde. The cells were analyzed on an EPICS 750 series flow cytometer (Coulter).

RNA Analysis. Total cellular RNA was extracted by use of guanidinium rhodanite following the protocol of Chirgwin *et al.* (20). Lytically infected OMK cells were harvested for RNA preparation when about 20% of the cell culture showed advanced cytopathic changes. RNA was separated on 1% agarose gels containing 2.2 M formaldehyde and blotted onto nitrocellulose filters. RNA transfer (Northern) blot hybridization was carried out at 40°C in buffer containing 50% formamide and 0.6 M NaCl/60 mM sodium citrate. Filters were washed finally in 30 mM NaCl/3 mM sodium citrate for 20 min at 65°C.

Transfection and Electrophoretic Transfer (Western) Blot Analysis. COS cells (10^6) were transfected with 5 μg of DNA using DEAE-dextran by the method of Cullen (21). Forty-eight hours later these cells were lysed, and $\approx 10^5$ cell equivalents was loaded into a 12% reducing acrylamide gel. In addition, infected cell lines were lysed, 10^6 live cell equivalents was loaded, and the proteins were electrophoresed and electroblotted onto nitrocellulose. Antiserum from HTLV-I-infected patients was allowed to bind to the filter overnight at room temperature and specific proteins were detected using an alkaline phosphatase-conjugated anti-human immunoglobulin and the appropriate substrates (Promega).

Immunoprecipitation. Immunoprecipitation of viral proteins produced by infected cells will be described in detail elsewhere (M.-C.D. and W.A.H., unpublished). Briefly, cells

were labeled overnight with [^{35}S]cysteine, washed, and lysed. The supernatants were then precipitated with preimmune rabbit serum prior to precipitation with rabbit antipeptide antiserum to remove nonspecific proteins. The infected cell lysates were then precipitated with the rabbit antipeptide antiserum specific for p27^{rex} and p21^{rex} (described in the text) that had been preabsorbed with uninfected, unlabeled cell lysates to remove nonspecific activity. The resulting immunoprecipitated proteins were analyzed by electrophoresis on a 12% reducing acrylamide gel followed by autoradiography.

Trans-Activation Assay. Cells (5×10^6) were transfected with 2 μg of the appropriate plasmid using DEAE-dextran as described (21). Forty-eight hours after transfection, the cells were counted and lysed, and 4×10^5 live cell equivalents was assayed unless otherwise noted for chloramphenicol acetyltransferase (CAT) activity. Results represent percentage conversion to the acetylated form of chloramphenicol after a 15-min enzymatic assay.

RESULTS

To examine the immortalizing potential of the X region of HTLV-I, apart from its demonstrated role in virus replication, it was necessary to introduce this region into a suitable target cell population in the absence of the replicative genes and in a form in which all potential X-region functions might be expressed. *H. saimiri* was selected as the vector as it is capable of infecting New World primate and human T lymphocytes (22, 37). Although *H. saimiri* is capable of transforming CD8$^+$ T cells (which also possess markers of the natural killer lineage) of some New World primates, repeated attempts to transform human lymphocytes, including cord blood lymphocytes, have been unsuccessful (ref. 16; R.G. and B.F., unpublished data). Nonetheless, as an additional assurance that the effect of X-region expression would be observed in the absence of *H. saimiri* transforming functions, the actual vector used for these experiments was the deleted *H. saimiri* strain 11S4 (23). The S4 deletion removes ≈ 3.5 kilobases (kb) of genetic information from the left terminal part of the (A + T)-rich region of the genome. The deleted virus S4 is capable of replication but is transformation-defective as judged by the failure of the virus to induce T-cell lymphomas in susceptible New World primates and by the failure to transform lymphocytes from these animals in tissue culture (24–27).

The sequences of HTLV-I used for these studies include all of the region between the 3' end of the envelope gene and the 3' long terminal repeat (LTR). A short region that overlaps the 3' end of the *pol* gene and the 5' end of the envelope gene was also included, as this region contains an exon that is joined by splicing to the X region. The entire 3' LTR is also present. The segment of the HTLV-I provirus used was obtained by deletion of HTLV-I *gag* and *pol* sequences and an internal deletion of envelope gene sequences. Some envelope sequences 3' to the splice donor and 5' to the end of the envelope gene were retained, as these sequences may affect expression of the first or second coding exons of *tax* and *rex* (for nomenclature review, see ref. 28). It is also possible that the sequences near the 3' end of the envelope gene are required for expression of information in the NCR region of X that contains several open reading frames that have the potential to encode proteins in addition to *tax* and *rex*. The fragments of the envelope gene that remain are predicted to be joined out of the correct reading frame. No products of envelope-related proteins were detected in experiments reported here (see below) or in other experiments with this plasmid (M.-C.D., K.M., and W.A.H., unpublished data).

The promoter used for expression of the HTLV-I proteins is the LTR of the murine retrovirus SL3-3. The SL3-3 promoter has been shown to direct high levels of RNA and protein synthesis in murine and human T cells (29). The promoter was placed 5' to the initiation codons of both p42^{tax}
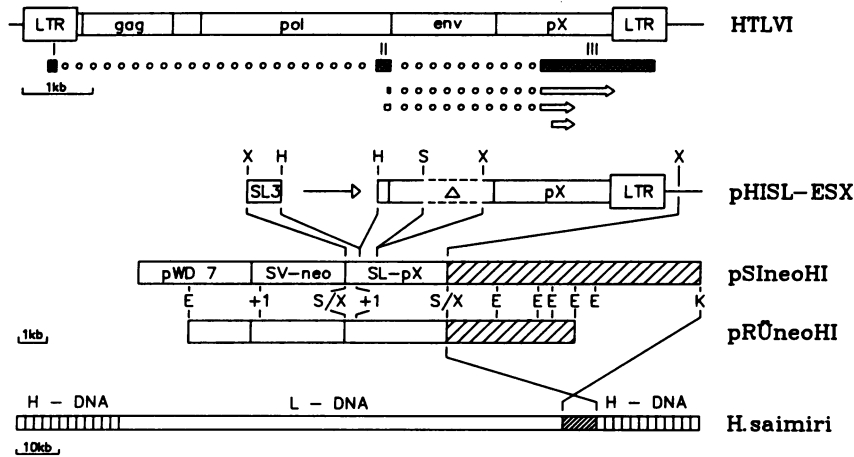
FIG. 1. Plasmids designed for the insertion of pX sequences into the genome of H. saimiri. The pX region of HTLV-I is transcribed into a doubly spliced mRNA (30, 31). The size and localization of the three exons are indicated. The proteins encoded by this transcript (p42^{tax}, p27^{rex}, and p21^{rex}) are shown as arrows. For expression cloning of the HTLV-I X region, a HindIII (nucleotide 5013) to Sac I fragment from a proviral clone (32), representing the 3' half of the virus was used. The majority of the env was deleted by a Sal I (nucleotide 5694) to Xho I (nucleotide 6518) deletion (SΔX). This HTLV-I sequence was fused to the SL3-3 (murine retrovirus) enhancer promoter contained in the Xho I (X) HindIII (H) fragment (29). The resulting expression cassette was shown to express p42^{tax} activity using pU3RI-CAT as an indicator. To insert the cassette into the unique Sal I (S) site of pRÜneo and pSIneo, the Sac I site was transformed into a Xho I site by use of T4 DNA polymerase and linkers. The plasmid pSIneo (37) contains the neo gene under the transcriptional control of the simian virus 40 early region promoter and the Kpn I-E fragment originating from the right L-DNA border of H. saimiri 11 (19). The plasmid pRÜneo is a deletion variant of pSIneo, lacking two EcoRI (e) fragments. The resulting plasmids were designated as pRÜneoH1 and pSIneoH1, respectively.

and p27^{rex}. Preparatory to introduction into the H. saimiri vector, the HTLV-I sequences were placed into the plasmids pRÜneo and pSIneo such that they were adjacent to H. saimiri sequences (Fig. 1). The viral DNA of H. saimiri strain 11S4 was cotransfected with the HTLV-I X plasmids onto OMK cells. The newly developed cloning strategy (R.G. and B.F., unpublished data) differed from earlier strategies to insert heterologous DNA as the heterologous sequences are inserted at the right terminus of the unique H. saimiri DNA (26). Recombinant virus was selected on OMK cells in the presence of the antibiotic G418, and plaques containing HTLV-I X DNA were identified by hybridization with radioactive DNA prepared from the HTLV-I X region. The recombinants were all found to contain the complete unrearranged HTLV-I X-region sequences by restriction enzyme and Southern blot analysis (not shown). Further restriction analysis of the recombinant H. saimiri genomes revealed no detectable rearrangements outside of the region where the recombinant events occurred (not shown). The H. saimiri recombinants express X-region RNA and p27^{rex} protein as well as p42^{tax} activity and protein upon lytic infection of OMK cells using antiserum raised to the nine carboxyl-terminal amino acids of the p27^{rex} protein and the ATLL patient antiserum, respectively (not shown).

The effect of infection by the H. saimiri-HTLV-I X recombinants on fresh human thymocytes and cord blood cells was examined. The cells were stimulated with PHA and, in the thymocyte experiment, phorbol ester (PMA) prior to infection and were grown in the presence of IL-2 after infection. In parallel, stimulated thymocytes were also cocultivated with mitomycin C-treated HTLV-I-producing cell lines [HUT 102 (1) and C91PL (7)]. A separate aliquot of the cells was also infected with the H. saimiri 11S4 deletion mutant. Continuous growth was observed in almost all thymus and in all cord blood cultures infected with the H. saimiri-HTLV-I X recombinants as well as in those populations cocultivated with HTLV-I-producing cells. All cultures have been maintained for a minimum of 36, and some 47, weeks after infection. In contrast, after 8 weeks, no viable cells were found in untreated control cultures or in cultures infected with the H. saimiri 11S4 virus. To determine whether the cell populations were dependent on IL-2, the cultures were split and a portion was maintained in the same medium lacking IL-2. None of the cultures remained viable for >10 days in the absence of IL-2. All thymocyte-derived cultures, including those infected with HTLV-I, have a doubling time of ≈30 hr. The doubling time for all of the cord blood cell cultures transformed by the H. saimiri recombinants is between 4 and 6 days at 30 weeks in culture.

The expression of lymphocyte markers was examined in these cell populations by using indirect immunofluorescence (Fig. 2). The amount of fluorescence was quantitated by flow cytometry. All cultures were found to express the T-cell surface markers CD4 and CD5 but were negative for CD8. In addition, all lines tested were found to express major histocompatibility complex class II (DR) molecules (not shown). All cultures were also found to express the receptor for IL-2 using an antibody specific for the 55-kDa α-chain (see Fig. 2, third horizontal panel).
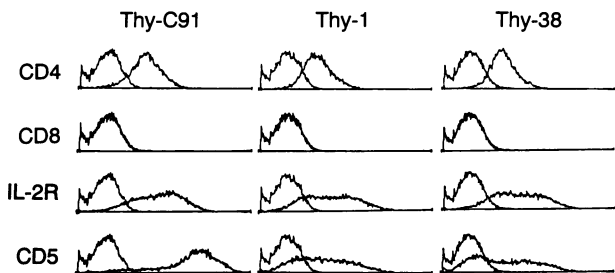

FIG. 2. Cell surface expression of T-cell markers in HTLV-I X-region transformed thymocyte lines. Lymphocyte lines were tested for cell surface expression of the indicated antigens using monoclonal antibodies. The fluorescein-conjugated second antibody alone was the negative control as depicted by the left-most peak in each panel. Fluorescence intensity is plotted on the abscissa versus cell number. Thy-C91 was derived from a cocultivation of normal thymocytes with the HTLV-I-infected line, C91PL. Thy-1 and Thy-38 are two lines derived from infection with H. saimiri HTLV-I X-region recombinants. Similar results were obtained with two other thymocyte lines, Thy-HUT (see Table 1) and Thy-40 (an additional line derived from infection with the recombinant II-40), as well as with two cord blood lymphocyte lines, Kati-40 and A-3. IL-2R, IL-2 receptor.
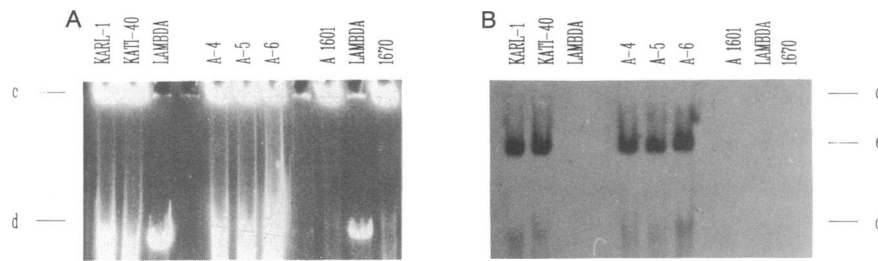
FIG. 3.   Episomal viral DNA persisting in transformed cord blood lymphocytes. Ten weeks after infection one million cells from continuously growing lymphocyte cultures with virus strains II-1 (Karl-1), II-40 (Kati-40), II-40 (A-4), III-22 (A-5), III-38 (A-6) were lysed carefully in the slot of a 0.8% agarose gel and subjected to electrophoresis (17). Two T-cell lines (22) containing episomal *H. saimiri* (1670) and *Herpesvirus atelas* DNA (A1601) were prepared as controls. Bacteriophage λ DNA, digested with HindIII, was used as a size marker. (A) Ethidium bromide-stained gel to localize the chromosomal DNA (c) and linear-degraded DNA (d). (B) Southern blot. The Gardella-type gel (17) was blotted onto a nitrocellulose membrane and annealed with a radioactive pSV2neo-DNA probe. The position of episomal DNA is indicated (e). Similar results to those shown for the cord blood lymphocyte lines were obtained for the thymocyte lines Thy-1, Thy-38, and Thy-40.

It is reported the *H. saimiri* DNA exists as episomal DNA in marmoset lymphoid tumor cell lines (18, 33). Examination of the thymocyte and cord blood cell lines using Gardella-type gels (17), which reveal the presence of episomal DNA after *in situ* lysis of the cells in the well of the gel, showed that the HTLV-I X sequences also exist as large episomes (150–200 kb) at high multiplicity (about 100 copies per cell) (Fig. 3). The size and number of genome copies per cell were estimated for the cord blood lines from comparison with *H. saimiri*-transformed tumor cell line 1670 (22).

Restriction enzyme digestion followed by Southern blot analysis revealed intact X-region DNA in the thymocyte and cord blood cell lines infected with the *H. saimiri* recombinants. Total RNA was extracted from these cultures and analyzed by using a radioactive probe to the HTLV-I X-region DNA. RNA was also extracted from HTLV-I-infected cell lines. RNA species corresponding to the full-length transcript, the mRNA encoding the envelope glycoprotein, and the mRNAs corresponding to the *tax* and *rex* products were detected as reported (30, 32, 34) in the HUT 102 cell line as well as in thymocyte lines derived by cocultivation with HUT 102 and C91PL. RNAs of ≈3.8 kb and ≈2.0 kb were detected in cells infected with the *H. saimiri*–HTLV-I X recombinants (Fig. 4). These species correspond to the full-length transcript initiated within the SL3-3 LTR and the major spliced product. The ratio of full-length transcript to spliced HTLV-I RNA is ≈10:1, as determined by densitometry tracing of one of the autoradiograms.

To determine whether any of the X-region functions were expressed in the *H. saimiri* recombinant transformed lymphocytes, the ability of these cells to express *tax* and *rex* proteins was analyzed. Western blot analysis using an ATLL patient antiserum demonstrated in Fig. 4B that the cell lines produce p42^{tax}. No truncated envelope protein product is detected in these cells by patient antiserum that recognizes the *env* proteins. To determine whether the *tax* product detected was active, several of the *H. saimiri*–HTLV-I X-infected lines and cultures cocultivated with HTLV-I were transfected with the indicator plasmid pU3R1-CAT (35) that contains the HTLV-I LTR located 5′ to the bacterial gene for CAT. The relative activity of the pU3R1-CAT and pRSV-CAT plasmids was determined in the Jurkat T-cell line that does not contain HTLV-I sequences. The results of the experiment summarized in Table 1 demonstrate that all of the *H. saimiri*–HTLV-I X-infected lines tested express active p42^{tax}. Immunoprecipitation with anti-*rex* antiserum revealed that the thymocyte cultures infected with the *H. saimiri*–HTLV-I X recombinants tested express p27^{rex} (Fig. 4). The cultures cocultivated with HUT 102 and C91PL also express p27^{rex} (Fig. 4, lane 1).
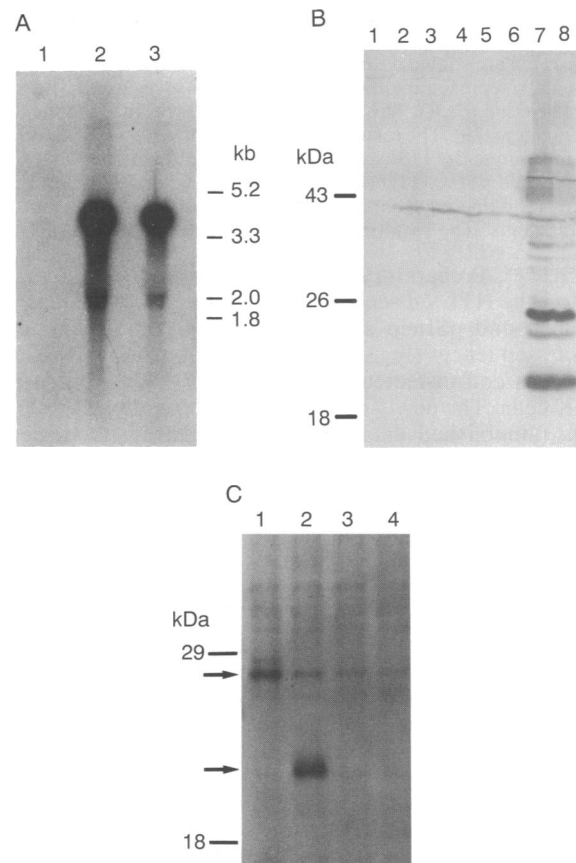

FIG. 4.   HTLV-I X-region expression in human T-cell lines transformed by the recombinants. (A) Transcription of the X region in transformed cord blood lymphocytes. RNA was extracted 13 weeks after infection. Fifteen micrograms of total cellular RNA from cell lines Jurkat (lane 1), Kati-40 (lane 2), and A-6 (lane 3) was separated on a denaturing gel, blotted onto nitrocellulose, and hybridized to an X DNA-specific radioactive probe. The positions of the 28S, 23S, 18S, and 16S rRNAs of human and *Escherichia coli* origin are indicated. Results similar to those shown were obtained with the thymocyte lines Thy-1, Thy-38, and Thy-40. (B) Western blot analysis of p42^{tax} protein expression in transformed thymocyte lines. COS cell controls were transfected with SV2neo, a negative control (lane 1); a *tax-*, *rex-*producing plasmid, EHX-1, driven by the human immunodeficiency virus promoter (lane 2); and a *tax-*, *rex-*producing plasmid driven by the HTLV-I LTR (lane 3). Infected cell lines were Thy-1 (lane 4), Thy-38 (lane 5), Thy-40 (lane 6), C91PL (lane 7), and Thy-C91 (lane 8). (C) Immunoprecipitation of p27^{rex} and p21^{rex} in transformed thymocyte lines. Arrows indicate the positions of p27^{rex} and p21^{rex}. Infected cell lines shown are Thy-HUT (lane 1), HUT 102 (lane 2), Thy-1 (lane 3), and Thy-38 (lane 4).

Table 1. *tax* activity in *H. saimiri*-HTLV-I-transformed cell lines

| Cell line | % conversion | | Ratio* |
	pU3R1-CAT	pRSV-CAT	
Jurkat	0.3	1.9	0.2
Thy-HUT[†]	76.5	0.8	96
Thy-1[‡]	76.7	3.0	25
Thy-38[§]	89.0	2.2	40
Thy-40[‡]	76.2	1.9	40
Kati-40[¶]	89.8	2.0	45

*pU3R1-CAT activity/pRSV-CAT activity.
[†]Thymocyte line derived from cocultivation with mitomycin C-treated HUT 102 cells.
[‡]Assayed for 4×10^4 live cell equivalents.
[§]Assayed for 10^5 live cell equivalents.
[¶]Cord blood lymphocyte line.

DISCUSSION

The results presented here demonstrate that the region of the HTLV-I virus present in the *H. saimiri*-HTLV-I X recombinants confers the ability to transform to continuous IL-2-dependent growth primary human lymphocytes derived from cord blood and thymus. The phenotype of the cells closely resembles that of ATLL tumor cells and of cells derived in culture by cocultivation with HTLV-I-producing cell lines. The *H. saimiri*-HTLV-I X-transformed human cells are CD4$^+$, CD8$^-$ and express high levels of IL-2 receptor. The infected cultures are capable of prolonged growth in the presence of IL-2. This report demonstrates a role of the X region in immortalization independent of its role in replication. The immortalizing ability of the HTLV-I X region does not require the *gag*, *pol*, or intact *env* proteins of the virus. These experiments also demonstrate that the X region determines the phenotype of the cell as it is unlikely that the HTLV-I and *H. saimiri* share the same surface receptor.

We think it unlikely that *H. saimiri*-encoded activities contribute directly to immortalization for the following reasons. *H. saimiri* does not transform human cord blood or thymic lymphocytes (16). The S4 deletion variant used for these experiments does not even transform New World primate lymphocytes. Moreover, the phenotype of the transformed cells is typical of HTLV-I-transformed cells and differs from the phenotype of New World primate cells transformed by the parental *H. saimiri* (36). The phenotype of the cells immortalized by the *H. saimiri*-HTLV-I X recombinant is precisely that of cells immortalized by HTLV-I.

These experiments do not address which of the potential X-region products specify the immortalizing functions. The cell lines do express the known X-region-encoded proteins, including *tax* and *rex*. In addition to those proteins, several other open reading frames in the X region exist that may specify additional proteins. The role of each of the potential X-region products in immortalization is now amenable to study using mutants introduced into the *H. saimiri* vector. The experiments also demonstrate the utility of the *H. saimiri* vector for studies of gene expression in human lymphocytes.

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