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Characterization and distribution of nucleic acid sequences of a novel type C retrovirus isolated from neoplastic human T lymphocytes

(RNA tumor virus/cutaneous T-cell lymphoma-leukemia/mycosis fungoides/molecular hybridization)

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ABSTRACT **A type C retrovirus (designated HTLV) recently isolated from a cell line derived from a lymph node and later from peripheral blood of a person with cutaneous T-cell lymphoma (mycosis fungoides) was characterized by nucleic acid hybridization experiments. HTLV [^{3}H]cDNA hybridized 90% to its own 70S RNA with kinetics consistent with the genetic complexity of other retroviruses, but it did not hybridize substantially to RNA or proviral DNA from any animal retroviruses (types B, C, and D), including those from nonhuman primates. Conversely, [^{3}H]cDNA from other retroviruses did not hybridize to RNA or DNA of the human T-cell line producing HTLV. HTLV proviral sequences were present (two to three copies per haploid genome) in DNA of these cells, and homologous sequences were present in the cell cytoplasmic RNA (0.3% viral sequences by weight). HTLV-related nucleic acid sequences were not found in DNA from various other human tissues. The results indicate that HTLV is a new class of type C virus that is not an endogenous (genetically transmitted) retrovirus in man.**

Retroviruses have been isolated from many species (1, 2), and some are involved in the etiology of naturally occurring leukemias, lymphomas, and sarcomas of animals (1–3). Virus-related components have been detected in human tissues (for reviews, see refs. 2 and 3) but in the rare instances in which replicating virus has been isolated, such isolates have been closely related to previously described primate viruses, especially simian sarcoma virus (SSV), and the possibility of laboratory contamination could not be precluded. Recently, a type C virus was isolated from a human histiocytic lymphoma which, although again related to viruses of the SSV/gibbon ape leukemia virus (GALV) group, shows apparent differences (4).

Detection of complete retrovirus particles is generally obtained only after *in vitro* cell proliferation (5), and in animal leukemias and lymphomas, T lymphocytes are often the target cells for virus infection (6). With rare exceptions, these cells could not be grown *in vitro* from human specimens, precluding the possibility of virus isolation. However, continuous *in vitro* growth of normal (7, 8) and neoplastic (9) human T lymphocytes was recently made possible by the finding of a growth factor, termed "T-cell growth factor (TCGF)" (7, 8), which has now been purified (10). Normal T cells require prior activation by antigen or phytohemagglutinin to develop TCGF receptors, after which they respond to TCGF (10). In contrast, purified TCGF produces continuous growth of neoplastic T cells directly, without initial stimulation (9).

We recently described the isolation of a type C virus, called "HTLV," from human neoplastic T lymphocytes cultured from blood and from a lymph node of a patient with cutaneous T-cell lymphoma (mycosis fungoides) (11). Electron microscopic examination and the presence of particulate media reverse transcriptase activity indicated that these cells were producing a type C virus. This reverse transcriptase activity was not inhibited by antisera to reverse transcriptases from a number of previously described retroviruses, including SSV [simian sarcoma-associated virus (SSAV)]/GALV and baboon endogenous type C virus (BaEV) (11).

Here we describe an analysis of the nucleotide sequences of HTLV. The sequence homologies of [^{3}H]cDNA and RNA of HTLV were compared to those of known animal retroviruses by molecular hybridization. In addition, the level of HTLV sequences (DNA and RNA) in the human T cells producing HTLV was assessed. Normal human DNA was tested for sequence homology with HTLV to determine whether HTLV is a germline-transmitted endogenous virus of humans or one that may be acquired after fertilization.

MATERIALS AND METHODS

Cells and Viruses. The T cells of origin for HTLV (designated HUT 102 and originating from the lymph node of the T-cell lymphoma patient), the methods of viral harvest and purification, and the methods for short-term (72 hr) culture of phytohemagglutinin-stimulated peripheral blood lymphocytes from pooled normal donors have been described (11–13). Malignant ovarian adenocarcinoma cells in ascites fluid were obtained from a paracentesis specimen. Additional normal human tissues were obtained from autopsy specimens. Animal tissues were obtained from HEM Research (Rockville, MD). HTLV was purified from clarified media of HUT 102 cells by centrifugation in a model K ultracentrifuge as described (11) and then further purified by equilibrium density gradient centrifugation (see *Results*). Other viruses were grown in the cell lines indicated in the appropriate figure and table legends. Avian myeloblastosis virus (AMV) was obtained from J. Beard (Life Sciences, Gainesville, FL).

Preparation of Nucleic Acids. Cellular DNA and RNA were prepared by Pronase/NaDodSO$_4$ digestion and organic solvent extraction as described (14). Where noted, poly(A)$^+$RNA was

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Abbreviations: dXTP, deoxyribonucleoside triphosphate; C_0t and C_rt, concentration (in mol/liter) of DNA and RNA, respectively, multiplied by time (in sec) of hybridization; SSV, simian sarcoma virus; SSAV, simian sarcoma-associated virus; GALV, gibbon ape leukemia virus; MuLV, murine leukemia virus; MuSV, murine sarcoma virus; FeLV, feline leukemia virus; FeSV, feline sarcoma virus; AMV, avian myeloblastosis virus; SMRV, squirrel monkey retrovirus; BaEV, endogenous baboon type C virus; BLV, bovine leukemia virus; MPMV, Mason–Pfizer monkey virus; TCGF, T-cell growth factor.

purified by oligo(dT)-cellulose chromatography (14). Viral 70S RNA was purified by Pronase/$NaDodSO_4$ digestion, phenol/chloroform extraction, and velocity gradient centrifugation as described (14). Unless otherwise indicated, viral [^{3}H]cDNA (20 $\times 10^6$ cpm/μg) was prepared in endogenous reverse transcriptase reactions with purified virions as described (15). Reaction mixtures contained 0.05% Nonidet P-40, 0.1 mM ^{3}H-labeled deoxyribonucleoside triphosphates ([^{3}H]dXTPs), 0.06 M KCl, 2 mM $MnCl_2$, 0.05 M Tris·HCl (pH 8.3), actinomycin D at 25 μg/ml, 20 mM dithiothreitol, and DNase-digested calf thymus DNA (2.4 mg/ml) as a random primer. SSV, GALV, and murine leukemia virus (MuLV) cDNAs protected ^{125}I-labeled viral RNA from RNase digestion 80–100% at cDNA/RNA ratios of 5:1. The reaction mixtures were incubated at 37°C for periods varied according to the kinetics of dXTP incorporation (acid-precipitable counts often decrease after maximal incorporation is achieved, probably due to adventitious nucleases). Higher-titered viruses, such as 71AP1 (SSV), could be incubated for 2 hr with no decrease in counts; however, lower-titered viruses such as HTLV reached a maximum in 20–25 min under the conditions used, at which time the reaction was terminated.

After termination of the reaction with $NaDodSO_4$, cDNA was purified by alkaline hydrolysis (0.3 M KOH, 10 min, 100°C), organic solvent extraction, and precipitation with cetyltrimethylammonium bromide as described (16). In some cases, [^{3}H]cDNA was prepared in reconstituted rather than endogenous reverse transcriptase reactions. In brief, for synthesis of Mason–Pfizer monkey virus (MPMV) and some bovine leukemia virus (BLV) cDNA preparations, viral 70S RNA (5 μg) was incubated for 1–2 hr at 37°C with 300 units of AMV reverse transcriptase (obtained from J. Beard, Life Sciences, Gainesville, FL) as described for the endogenous reactions but with 6 mM $Mg(OAc)_2$ instead of $MnCl_2$ and with Nonidet P-40 present at 0.01%. 70S RNA and [^{3}H]cDNA from squirrel monkey retrovirus (SMRV) (17), from murine mammary tumor virus (MMTV), and, in some cases, from MPMV were kindly provided by J. Schlom (National Cancer Institute). Some BLV cDNA and 70S BLV preparations were gifts from A. Burny (Free University of Brussels, Belgium).

Molecular Hybridization. Hybridization of [^{3}H]cDNA to RNA was in 50% formamide/0.45 M NaCl/0.045 M sodium citrate, pH 7, at 37°C. RNA concentrations were as indicated in the text or figure and table legends. C_rt values (concentration of RNA times duration of hybridization, mol·sec/liter) (18) were not corrected for salt, temperature, or solvent. Hybridization of viral [^{3}H]cDNA to cellular DNA was carried out in 0.4 M NaCl/10 mM Tris·HCl, pH 7.4, at 65°C with at least a 10^7-fold weight excess of driver DNA. C_0t values (concentration of DNA times duration of hybridization, mol·sec/liter) were corrected to standard conditions (19). Hybridization was assayed by digestion with single strand-specific nuclease S1 from *Aspergillus oryzae* (Miles) as described (14). Rehybridization of cell DNA was assayed by hydroxyapatite chromatography (19) at 65°C as described for Fig. 2. Hybridization of ^{125}I-labeled RNA to DNA was carried out at $>10^7$-fold weight excess of DNA in 0.4 M phosphate (pH 7) at 65°C and assayed by digestion with RNase A as described (14).

Isolation of HTLV 70S RNA. HTLV concentrated from media from the HUT-102 line by rate zonal centrifugation on the model K ultracentrifuge was used for the preparation of HTLV 70S RNA. After Pronase/$NaDodSO_4$ digestion and extraction with phenol/chloroform, the RNA was bound to a column of oligo(dT)-cellulose (0.5 g) (T1, Boehringer Mannheim) in 0.5 M LiCl. The column was washed with 0.5 M LiCl until the optical absorbance of the eluate reached background. The poly(A)$^+$ RNA was eluted with 8 ml of 10 mM Tris, pH 7.4/0.4 M in LiCl, rechromatographed as above, precipitated with ethanol/salt, and further purified by velocity gradient centrifugation in an SW 41 rotor (10–30% glycerol, 4 hr, 40,000 rpm, 4°C). A distinct peak of RNA that sedimented at about 70S was evident. After ethanol/salt precipitation, approximately 10 μg of RNA was recovered from 45 liters of media. The purified 70S RNA had a 260 nm/280 nm absorbance ratio of 2.0. [^{3}H]Uridine-labeled HUT 102 media also gave a 70S peak of poly(A)$^+$ [^{3}H]RNA (data not shown).

RESULTS

Characteristics of HTLV [^{3}H]cDNA Probe. Concentrated, purified HTLV from the model K ultracentrifuge was rebanded to equilibrium in sucrose density gradients (22–55% sucrose in TNE) in an SW 41 rotor (4°C, 16 hr, 22,000 rpm). The gradient was assayed for DNA polymerase activity with oligo(dT)$_{12-18}$·poly(A) and oligo(dT)$_{12-18}$·poly(dA) as primer-templates. A peak of DNA polymerase activity was present at 1.16 g/cm^2 with marked preference for oligo(dT)·poly(A) over oligo(dT)·poly(dA), a characteristic of reverse transcriptase (20). Most of the activity with oligo(dT)·poly(dA), likely representing residual cellular DNA polymerase activity, bands at higher density. The peak fractions of reverse transcriptase activity were pooled, concentrated by velocity gradient centrifugation (100,000 $\times$ g, 1 hr), and used for the synthesis of [^{3}H]cDNA as described above.

The yield of [^{3}H]cDNA prepared from HTLV purified as described above from 5 liters of media was generally 150,000–200,000 cpm (2×10^7 cpm/μg). After hydrolysis of residual RNA with KOH (0.3 M, 100°C, 10 min), the resistance of [^{3}H]cDNA to nuclease S1 ranged from 5% to 12%. HTLV cDNA hybridized 90% to HTLV 70S RNA with a $C_rt_{1/2}$ of 0.15 (Fig. 1). This $C_rt_{1/2}$ value is close to values obtained in the same solvent system for other type C viruses (14, 21) and indicates that the HTLV 70S RNA is reasonably homogeneous with respect to viral sequences. The observed hybridization thus is not likely due to a minor RNA species. This is further demonstrated by the specificity of hybridization of ^{125}I-labeled HTLV 70S RNA to cell DNA (see below).

Lack of Sequence Homology to Other Retroviruses. The relatedness of HTLV to other retroviruses was assessed by molecular hybridization. HTLV [^{3}H]cDNA was hybridized, to a $C_rt \geq 2$, to 70S RNA from various retroviruses under condi-

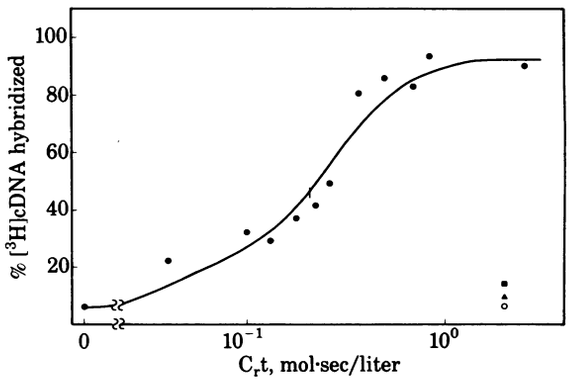

FIG. 1. Kinetics of hybridization of HTLV [^{3}H]cDNA to 70S RNA. [^{3}H]cDNA was hybridized to the indicated C_rt with 0.8–1.2 μg of 70S RNA. Hybridization was assayed by digestion with nuclease S1. The percentage hybridization was not normalized. ●, HTLV 70S RNA; ■, SSV(SSAV) 70S RNA; ▲, MuLV-R 70S RNA; ○, AMV 70S RNA.

Table 1. Lack of homology between RNA of HTLV and other retroviruses

Source of viral RNA		% hybridization with HTLV [^{3}H]cDNA
Virus	Cell line for production	
HTLV	HUT 102 (human)	90
SSV(SSAV)	71AP1 (marmoset)	16
SSV(SSAV)	A204 (human)	11
SSAV	NRK (rat)	11
$GALV_H$	6G-1 (gibbon)	13
BaEV(M7)	A204 (human)	7
SMRV	Cf2th (dog)	6
MPMV	NC37 (human)	3
BLV	FLK (lamb)	11
MMTV	(RIII milk)	8
$MuLV_{AKR}$	NIH 3T3 (mouse)	10
$MuLV_R$	BALB 3T3 (mouse)	7
$MuLV_{HIX}$	F3C17 (mink)	8
$MuSV_{Moloney}$	(mouse spleen)	9
$MuSV_{Kirsten}$	NRK (rat)	8
$FeLV_{Rickard}$	FEF (cat)	8
$FeSV(FeLV)_{ST}$	MSTF (mink)	7
AMV	(chicken plasma)	4
RD114	RD (human)	3

[^{3}H]cDNA and 70S RNA were prepared and hybridized in 50% formamide/0.45 M NaCl, pH 7; 500–1000 cpm of [^{3}H]cDNA was hybridized to 0.8–1.2 μg of 70S RNA to a C_rt of >2 and then assayed with nuclease S1 as described (14). Cell lines used for virus growth included: HUT 102, the cell line producing HTLV (11); 71AP1, a marmoset tumor line producing SSV(SSAV); A204, human rhabdomyosarcoma cell line; NRK, normal rat kidney; 6G-1, a leukemic gibbon lymphoblast line; CCL88, bat lung fibroblast line; BALB and NIH 3T3, mouse fibroblast lines; F3C17, a mink fibroblast clonal line nonproductively transformed with feline sarcoma virus (FeSV), Synder–Theilen strain; FEF, feline embryo fibroblasts; MSTF, a mink clonal line transformed with FeSV and superinfected with feline leukemic virus (FeLV); NC37, a human lymphoblast line; FLK, fetal lamb kidney fibroblasts; and Cf2th, a canine thymus fibroblast line. $MSV_{Moloney}$ was purified from infected mouse spleen, MMTV was from infected mouse milk, and AMV was from infected chicken plasma. $GALV_H$ is the Hall's Island isolate of GALV (14), $GALV_{SF}$ is the San Francisco strain of GaLV (22), and $MuLV_{AKR}$, $MuLV_R$, and $MuLV_{HIX}$ are the AKR, Rauscher, and HIX strains of MuLV.

tions of moderate stringency. Because 70S RNA was not available for some endogenous viruses, the HTLV cDNA was hybridized instead to host tissue or cell DNA, which contains multiple endogenous virogene copies, or to DNA from cell lines infected with virus.

No significant hybridization of HTLV cDNA was observed with 70S RNA from several strains of MuLV, MuSV, FeLV, and FeSV or from BLV, MMTV, SMRV, MPMV, RD114, BaEV, or AMV (Table 1). Very low hybridization (≈5% above background) was consistently observed with RNA from different strains of GALV and SSAV, suggesting that HTLV is only slightly related or not related at all to viruses of this group. In addition, 70S RNA from HTLV did not significantly compete the hybridization of ^{125}I-labeled RNA from SSAV to SSAV proviral DNA (not shown). Also, SSAV strong-stop cDNA, homologous to the 5′ 145 nucleotides of SSAV viral RNA, did not hybridize to HUT 102 RNA or DNA.

No hybridization was observed with DNA from cells or tissues of different species possessing endogenous virogenes (including owl monkey, macaque, capuchin, langur, baboon, colobus, guinea pig, pig, mink, hamster, rat, and cat) or from calf thymus or BLV-infected sheep cells (Table 2). Reciprocal experiments were conducted using [^{3}H]cDNA from SSV(SSAV),

Table 2. Lack of hybridization of HTLV cDNA to mammalian DNAs with known endogenous virogenes

DNA from	% [^{3}H]cDNA hybridized
HUT 102	45
Langur (spleen)	6
Owl monkey (OM-1)	7
Macaque (spleen)	10
Colobus (brain)	7
Baboon (liver)	8
Guinea pig (GP-1)	5
Pig (CCL33/PK)	5
Sheep (FLK/BLV)	3
Mink (CCL64)	2
Hamster (HEF/HaLV)	12
Rat (NRK)	2
Cat (placenta)	5
Calf (thymus)	7

HTLV cDNA hybrid formation was performed in 0.4 M NaCl at 65°C; 500–1000 cpm of cDNA was hybridized to 600 μg of DNA to a C_0t of 20,000 (corrected). Hybridization was assayed by nuclease S1 digestion as described (14). Hybridization values with DNA were corrected for 0-time values but were not otherwise normalized. Cell lines were as follows: OM-1, owl monkey fibroblasts producing the endogenous owl monkey virus (23); GP-1, guinea pig fibroblasts producing the endogenous guinea pig virus (24); CCL33/PK, pig kidney fibroblasts producing the endogenous pig virus (25); HEF/HaLV, hamster embryo fibroblasts producing the endogenous hamster virus (26); CCL64, mink lung fibroblasts containing the endogenous mink virus (27); NRK, normal rat kidney fibroblasts containing endogenous rat virus (28); FLK/BLV, fetal lamb kidney fibroblasts infected with BLV (29, 30). Langur tissue contains multiple virogenes for the langur endogenous virus (31).

MPMV, SMRV, BLV, MMTV, and BaEV and comparing the level of hybridization to the homologous RNA and to RNA and DNA from HTLV-infected cells. Although all these [^{3}H]cDNAs hybridized >60% to their respective 70S RNAs, no hybridization was detectable with cytoplasmic RNA (Table 3) or cell DNA (data not shown) from the cell line producing HTLV, both of which contain readily detectable HTLV sequences. This indicates that HTLV is not significantly related to a wide variety of retroviruses.

Analysis of HTLV Nucleotide Sequences in Cells Producing HTLV. HTLV cDNA was hybridized to cytoplasmic RNA and cell DNA purified from HUT 102, the cell line producing HTLV. HTLV cDNA hybridized extensively to HUT 102 cy-

Table 3. Specificity of viral sequences in RNA from the cell line producing HTLV

[^{3}H]cDNA from	% hybridization to	
	HUT 102 (HTLV) cytoplasmic RNA	Homologous 70S RNA
HTLV	65	90
SSV(SSAV)	12	98
BaEV	12	90
SMRV	7	75
MPMV	7	62
BLV	10	62
MMTV	14	70

[^{3}H]cDNA was hybridized to 200 μg of HUT 102 (HTLV) cytoplasmic RNA to a C_rt of >400 or to 0.8–1 μg of 70S RNA to a C_rt of >2, and the hybridization was assayed by nuclease S1 digestion as described for Table 1. SSV(SSAV) was grown in the 71AP1 cell line. Other viruses were grown in the cell lines indicated in Table 1. Homologous 70S RNA refers to the RNA from the same virus used to generate the [^{3}H]cDNA.

Table 4. Lack of HTLV sequences in normal human DNA and RNA

Source of nucleic acid	% [^{3}H]cDNA hybridized to DNA	RNA
HUT 102 (HTLV)	45	58
Normal human tissues:*		
PHA-stimulated lymphocytes	10	9
Spleen	3	ND
Liver	7	ND
Kidney	6	ND
Brain	3	ND
Human ovarian adenocarcinoma†	7	ND
Canine thymus fibroblasts (Cf2th)	5	8

HTLV [^{3}H]cDNA was prepared and hybridized to 200 μg of cytoplasmic RNA or 600 μg of cell DNA, and the hybridization was assayed as described for Tables 1 and 2. RNA and DNA from Cf2th, a canine thymus fibroblast line, were used for nonhuman controls. ND, not determined. Hybridization values are corrected for 0-time values but are not otherwise normalized.

* Human tissues included peripheral blood lymphocytes pooled from 20 normal donors, stimulated with phytohemagglutinin in short-term culture and purified on a nylon column; spleen from a patient who died of pulmonary embolism; liver from a patient who died of sickle cell anemia; kidney from a patient who died of myocardial infarction; and brain from a patient who died as a result of hypertrophic heart.

† Ovarian adenocarcinoma paracentesis specimen, consisting primarily of tumor cells.

toplasmic RNA (Table 4). The $C_rt_{1/2}$ was 40 (not shown), indicative of a viral RNA content of about 0.3% by weight of total cytoplasmic RNA, similar to other retrovirus-infected cells (21, 32). Cytoplasmic poly(A)$^+$RNA fractionated on oligo(dT)-cellulose was enriched for HTLV sequences (not shown), although they were also detectable in poly(A)$^-$RNA.

HTLV sequences in HUT 102 cell DNA were detectable with as little as 100 μg of DNA. With 200 μg of HUT 102 DNA the maximal hybridization was 45–50%, and it could be increased to 60% with 600 μg of DNA. The copy number of these viral sequences was determined by comparing the kinetics of hybridization of HTLV cDNA to HUT 102 DNA (assayed by nuclease S1 digestion) with the rehybridization of unique copy HUT 102 DNA (assayed by hydroxyapatite chromatography). At low C_0t values, approximately 38–40% of the cell DNA is rehybridized (Fig. 2A). At higher C_0t values, the double-stranded cell DNA increased to about 80%. The $C_0t_{1/2}$ of this reaction, which represents the rehybridization of unique-copy DNA, was about 2200, which is in agreement with literature values for primate unique-copy DNA (33). The $C_0t_{1/2}$ of the HTLV cDNA/HUT 102 DNA reaction was about 1000, suggesting that the HTLV proviral sequences are present at two to three copies per haploid genome of HUT 102 (assuming the kinetics of hybridization are similar when assayed by both methods).

Absence of HTLV Virogenes from Normal Uninfected Human Cells. Cytoplasmic RNA (poly(A)$^+$ and poly(A)$^-$) purified from normal, fresh, peripheral blood human T lymphocytes, pooled from multiple donors and phytohemagglutinin-stimulated in short-term (72 hr) culture, contained no detectable HTLV sequences (Table 4). These results indicate that HTLV cDNA is specific for HTLV sequences (in agreement with results presented above) and does not represent sequences homologous to human normal T-cell RNA.

The question of whether or not HTLV is an endogenous genetically transmitted virus of humans, present as a provirus but not normally expressed into RNA in human cells, was explored by analyzing various human DNA samples for HTLV DNA sequences. DNA from a human (A204) rhabdomyosarcoma cell line infected with the M7 strain of BaEV was also tested for HTLV-related sequences. The BaEV-related sequences, present at three to four copies per haploid genome (32), served as an internal control for DNA quality and for hybridization kinetics to sequences present at a relatively low copy number. The $C_0t_{1/2}$ of the reassociation of unique sequence DNA was the same as for HUT 102 (2100–2200) (Fig. 2B). BaEV cDNA hybridized to A204 (M7) DNA with a $C_0t_{1/2}$ of 700 (assayed by nuclease digestion S1), indicating the presence of about three copies of BaEV per haploid genome, in agreement with published results (32). In contrast, HTLV cDNA did not hybridize detectably to this DNA, even at an input of 600 μg of DNA and at C_0t values $>10^4$. Therefore, A204 cell DNA does not contain

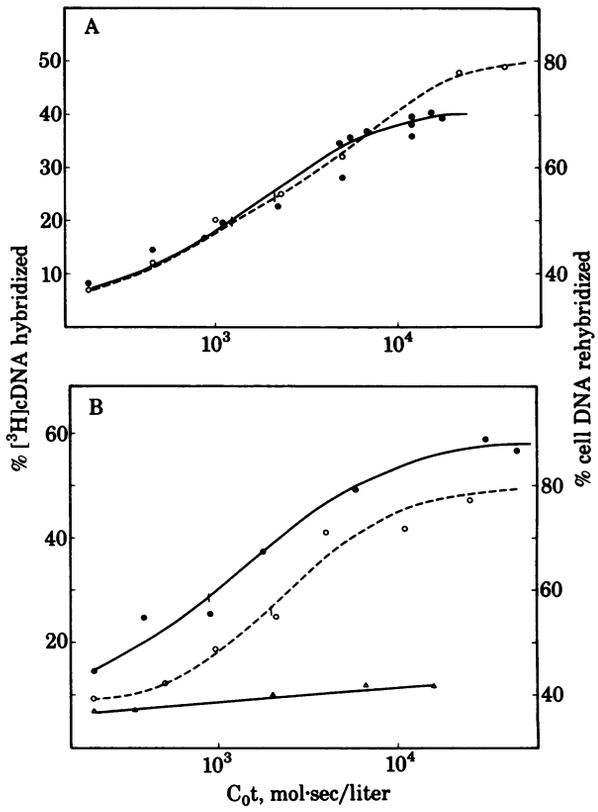

FIG. 2. Kinetics of hybridization of HTLV [^{3}H]cDNA to human DNA. [^{3}H]cDNA was hybridized with cell DNA and assayed for hybrid formation by nuclease S1 digestion. Hybridization values were not normalized. Cell DNA was also self-hybridized in 0.4 M NaCl (65%) to the indicated corrected C_0t value and adsorbed to hydroxyapatite (Bio-Rad, DNA grade) (bed volume, 1 ml) in 0.05 M phosphate, pH 7/0.6 M NaCl at 65°C. The column was washed with this buffer until the optical absorbance at 260 nm reached background levels. Single-stranded DNA was eluted with 2-ml portions of 0.14 M phosphate, pH 7/0.6 M NaCl, and the optical absorbance at 260 nm was determined for each fraction. When the absorbance reached background levels, double-stranded DNA was eluted with 0.4 M phosphate, pH 7/0.6 M NaCl and the absorbance was determined. The percentage rehybridization of cell DNA was calculated from the percentage of the total optical absorbance bound to the column that was eluted with the 0.4 M phosphate buffer. (*A*) [^{3}H]cDNA from HTLV hybridized to HUT 102 cell DNA. ●, Hybridization of [^{3}H]cDNA; ○, self-hybridization of HUT 102 cell DNA. (*B*) [^{3}H]cDNA from BaEV, strain M7 (●) or HTLV (△) was hybridized to cell DNA from human rhabdomyosarcoma (A204) cells experimentally infected with M7. ○, Self-hybridization of A204 (M7) cell DNA.

HTLV sequences or contains significantly less than one copy of HTLV viral sequences per haploid genome. To substantiate further that less than one copy of HTLV sequences is present per haploid genome in human DNA, HTLV cDNA was hybridized to DNA from several normal non-neoplastic human tissues and an ovarian adenocarcinoma paracentesis specimen. HTLV cDNA failed to hybridize significantly to any of these DNA samples, even at high DNA inputs and C_0t values (Table 4), again indicating that HTLV sequences are not ubiquitous in DNA from human cells or are only present at levels far less than one copy per haploid genome.

In order to substantiate that HTLV sequences are not endogenous to human DNA, 70S RNA from HTLV was labeled with ^{125}I (5×10^7 cpm/μg) and hybridized to a vast ($>10^7$-fold) weight excess of DNA from HUT 102 or to normal human DNA. The results are similar to those with cDNA: the RNA hybridized to HUT 102 DNA 40% by a C_0t of 20,000 (corrected) but only 8–9% to normal human DNA (data not shown). These results clearly indicate that HTLV is not a vertically transmitted virus endogenous to germ-line DNA of all humans.

DISCUSSION

HTLV, a retrovirus recently isolated in this laboratory (11) from a malignant T-cell line from an affected lymph node of a man with cutaneous T-cell lymphoma, has been partially characterized in molecular hybridization experiments. HTLV [^{3}H]cDNA hybridizes 90% to homologous 70S RNA with a $C_rt_{1/2}$ of approximately 0.15, indicative of a genetic complexity similar to that of other type C viruses. In contrast, HTLV cDNA hybridizes at most only slightly to 70S RNA from SSV(SSAV) or GALV and does not hybridize detectably to 70S RNA from other retroviruses or to DNA from tissues or cells of various animal species whose genomes contain multiple copies of endogenous virogenes. In addition, [^{3}H]cDNA from various animal retroviruses does not hybridize to RNA or to DNA of the cell line producing HTLV. HTLV nucleotide sequences are therefore not significantly related to any of these previously described retroviruses. We conclude that HTLV is a new retrovirus.

The cell line which produces HTLV contains two to three copies of HTLV provirus per haploid cell genome. The provirus thus is not present in the multiple copies (10–200 per haploid genome) typical of endogenous retroviruses (28, 31–33). Proviral sequences are expressed into RNA at levels (0.2–0.3% of total cytoplasmic RNA) typical of other retrovirus-infected cells (21, 32). HTLV-related sequences are not detectable in DNA from several other human cell lines and normal tissues. The probes and hybridization conditions used would easily detect these sequences even at levels below one copy per haploid genome. High levels of HTLV proviral sequences thus do not appear to be widely distributed in humans, although we cannot rule out the widespread presence of these sequences at less than 1 copy per 10 haploid genomes. We conclude, therefore, that HTLV is not an endogenous germ-line-transmitted virus ubiquitous in man; it either enters human cells by some type of infectious event which may be uncommon or is transmitted in the germ line of a few families (although the present data do not prove a human origin for HTLV). It is important to determine the distribution of HTLV in humans by a more extensive molecular hybridization survey, by assays of sera for antibodies, and by assays of cells for viral antigens to see whether the presence of HTLV will correlate with any human T-cell neoplasias.

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