JOURNAL OF VIROLOGY, June 1981, p. 906-915
0022-538X/81/060906-10$02.00/0

Vol. 38, No. 3

Immunological Properties of a Type C Retrovirus Isolated from Cultured Human T-Lymphoma Cells and Comparison to Other Mammalian Retroviruses

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Received 28 July 1980/Accepted 6 March 1981

HTLV strain CR ($HTLV_{CR}$) is a retrovirus which was isolated from a human T-cell lymphoma cell line. A protein of molecular weight 24,000, p24, was purified from this virus. Several results indicate that this p24 is an internal core protein of $HTLV_{CR}$. (i) The p24 copurified with viral cores. (ii) It was labeled with ^{125}I after disruption of the virus, but not when undisrupted virus was iodinated. (iii) The amount of p24 was directly proportional to the amount of $HTLV_{CR}$. (iv) In chromatographic properties, the $HTLV_{CR}$ p24 behaved similarly to the major structural protein (24,000- to 30,000-molecular-weight protein) of other retroviruses. A rabbit antiserum raised against disrupted $HTLV_{CR}$ precipitated the labeled p24, and the precipitation was competed for by unlabeled $HTLV_{CR}$ and by cytoplasmic proteins from cells producing $HTLV_{CR}$, but not by proteins from normal human cells, including normal growing human T-cells, and several cultured human cutaneous T-cell lymphoma lines. Proteins from several mammalian type B, type C, and type D viruses also failed to compete in this precipitation. Moreover, $HTLV_{CR}$ did not react in homologous and interspecies assays for p30 antigens of several mammalian type C and type D viruses. These observations agree with immunological comparisons between reverse transcriptase of $HTLV_{CR}$ and other retroviruses and nucleic acid sequence homology studies which indicate that the various $HTLV_{CR}$ isolates represent new retroviruses found in some human T-cell neoplasias.

The cellular DNA of several Old World and New World primates contains genetically transmitted information of either type C or type D retroviruses (for recent reviews, see references 6 and 10). The isolation of virus particles from cells of these primate species is a low-frequency event. Retroviruses are also associated with naturally occurring leukemias and sarcomas of gibbon apes and several lower animals (for recent reviews, see references 6 and 10). There have been several studies suggesting the presence of retroviral information in humans (for recent reviews, see references 8 and 9), but the actual isolation of retroviruses from human tissues has been difficult, and some of the putative isolates have been shown to be closely related to or indistinguishable from the infectious primate type C retrovirus group composed of simian sarcoma virus (SSV) and gibbon ape leukemia virus (GALV) (7, 13, 16, 18).

Recently, the isolation of a type C retrovirus, HTLV strain CR ($HTLV_{CR}$), was reported from the cultured cells (HUT102) of a patient with cutaneous T-cell lymphoma (mycosis fungoides) (11, 16a). Originally, the production of HTLV required induction with iododeoxyuridine, but the cells subsequently became constitutive producers after passage 56. The physical and biochemical properties of the isolated virus particles (16a), nature of the reverse transcriptase activity (H. M. Rho, B. J. Poiesz, F. W. Ruscetti, and R. C. Gallo, Virology, in press), and nucleic acid hybridization characteristics (16b) have been recently described. The present study was undertaken to ascertain any immunological relatedness that this virus might have to other known mammalian type B, type C, and type D retroviruses. Since p30 is the most conserved antigen in retroviruses in general the plan was to isolate the p30 equivalent of $HTLV_{CR}$ and to use it in the immunological assays. Our results described below indicate that $HTLV_{CR}$ is unique, and that there is no significant cross-reactivity between $HTLV_{CR}$ and other known retroviruses tested.

MATERIALS AND METHODS

Viruses, viral proteins and cells. Cells producing

HTLV$_{CR}$ were grown in RPMI 1640 containing 20% fetal calf serum. HTLV$_{CR}$ was concentrated from cell culture supernatant fluids by continuous-flow centrifugation, and the virus was purified further by equilibrium density banding in a 25 to 60% sucrose gradient. Normal human T-cells were grown in medium supplemented with T-cell growth factor as previously described (15, 17). Other viruses and viral antigens used in this study were the following. Baboon endogenous virus (BaEV-M7) grown in human rhabdomyosarcoma cells (A204) and Rauscher murine leukemia virus (R-MuLV) grown in JLSV-9 cells were obtained through the resources and logistics, National Cancer Institute; feline leukemia virus (FeLV) was grown in FL74 cells; Mason-Pfizer monkey virus (MPMV) and squirrel monkey retrovirus (SMRV) were grown in dog thymus cells; and SSV/simian sarcoma-associated virus (SSAV) was grown in marmoset cells (71AP1). Purified guinea pig virus (GPV) p26, owl monkey virus (OMC-1) p30, deer kidney virus (DKV) p30, viper retrovirus (VRV) p24, and the antibodies against GPV, DKV, OMC-1, VRV, BALB-2 virus, reticuloendotheliosis virus, and MPMV were kindly provided by M. Barbacid and S. A. Aaronson, National Cancer Institute. Murine mammary tumor virus (MMTV) p28 and antibody to *Mus cervicolor* mammary tumor virus were kindly provided by J. Schlom, National Cancer Institute. Macaque virus and langur virus were kindly provided by G. Todaro, National Cancer Institute. Bovine leukemia virus (BLV), grown in fetal lamb kidney cells, was purchased from Electronucleonics, Ltd. BLV p24 and antibody to BLV p24 were provided by A. Burny of Brussels, Belgium.

Purification of viral proteins. The major structural proteins of representative type C and type D viruses were purified by methods described previously (5, 19; P. D. Markham, F. W. Ruscetti, V. S. Kalyanaraman, L. Ceccherini-Nelli, N. R. Miller, M. S. Reitz, S. Z. Salahuddin, and R. C. Gallo, Cancer Res., in press). HTLV$_{CR}$ p24 was purified from HTLV$_{CR}$ banded twice in a sucrose gradient as follows. The virus was disrupted with 0.5% Triton X-100 and 0.8 M NaCl in buffer A (20 mM Tris-hydrochloride [pH 7.5], 20% glycerol, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride). After stirring in ice for 1 h, the solution was centrifuged at 35,000 rpm for 60 min in a Beckman Spinco type 40 rotor. The supernatant was dialyzed against buffer A to lower the salt concentration to 0.3 M and freed of nucleic acids by passage through DEAE-cellulose at 0.3 M NaCl in buffer A. The unadsorbed protein fraction was dialyzed against buffer B (10 mM *N,N*-bis-hydroxyethylaminoethanesulfonic acid, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride [pH 6.5]) and chromatographed on a 5-ml column of phosphocellulose equilibrated with the same buffer. The column was then developed with a 100-ml 0 to 0.6 M NaCl linear gradient in buffer B by collecting 1.5-ml fractions. The proteins of the gradient fractions were analyzed by electrophoresis in a 12% polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS) (14). The HTLV$_{CR}$ p24 eluted as a nearly homogeneous peak between 150 and 250 mM NaCl. The fractions containing HTLV$_{CR}$ p24 were pooled, concentrated by lyophilization, and were further purified by passing through an LKB Ultrogel AcA 54 column equilibrated with buffer C (10 mM sodium phosphate [pH 7.5], 10% glycerol, 0.1 mM phenylmethylsulfonyl fluoride).

Iodination of viral proteins. Purified proteins of HTLV$_{CR}$ and other type C and type D viruses were labeled with ^{125}I to specific activities of 5 to 20 μCi/μg by the chloramine-T method (12). The HTLV$_{CR}$ (50 μg) was labeled in a 100-μl reaction mixture containing 1% SDS, 10 μg of chloramine-T, and 500 μCi of Na^{125}I. Only 2 μg of chloramine-T was used when the labeling was done with intact virus.

Radioimmunoassays. Radioimmunoassays were performed by the double-antibody method described elsewhere (Markham et al., in press). Serial twofold dilutions of antisera were incubated with the labeled antigen (approximately 15,000 cpm) for 2 h at 37°C and further incubated overnight at 4°C. These samples were in a buffered solution containing 200 mM NaCl, 20 mM Tris-hydrochloride (pH 7.5), 0.3% Triton X-100, 1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride in a final volume of 200 μl. A 30-fold excess of the appropriate second antibody was then added and further incubated for 2 h at 37°C followed by 1 h at 4°C. The incubation mixtures were diluted to 1 ml with the buffer and centrifuged at 8,000 rpm for 4 min, and the percent radioactivity bound in the pellets was determined in an LKB Ultrogamma counter. In competition radioimmunoassays, unlabeled antigen was preincubated for 1 h at 37°C with a limiting dilution of the immune serum capable of precipitating 30% of the labeled antigen before addition of the labeled antigen. Further steps of the procedure were the same as described above for the standard radioimmune precipitations.

RESULTS

Labeled polypeptides of HTLV$_{CR}$. HTLV$_{CR}$ was banded twice in sucrose gradients (density, 1.16 g/ml) after concentrating by pelleting through a 30% glycerol cushion. When this material was labeled with ^{125}I under mild conditions in the absence of detergents and the profile of labeled proteins was examined by SDS-polyacrylamide gel electrophoresis, three prominent proteins were evident with molecular weights (mol wt) of 55,000, 45,000, and 13,000 (Fig. 1A). Whether all these proteins were of viral origin or whether any one of them corresponded to the envelope glycoprotein of the virus could not be determined. When the virus was initially lysed with 1% SDS and subsequently exposed to ^{125}I, three additional proteins appeared to be labeled (Fig. 1B). These patterns were similar to other retroviruses in that a large envelope glycoprotein, usually 50,000 to 80,000 in mol wt, and a low-mol-wt protein, p15, were labeled in intact virions under the above conditions, whereas 10,000-, 12,000-, 15,000-, 30,000-, and 70,000-mol-wt proteins were labeled when the disrupted viruses were used for labeling by the above method. The 24,000- and 18,000-mol-wt proteins (p24 and p18, respectively) were inaccessible to labeling until the virions were

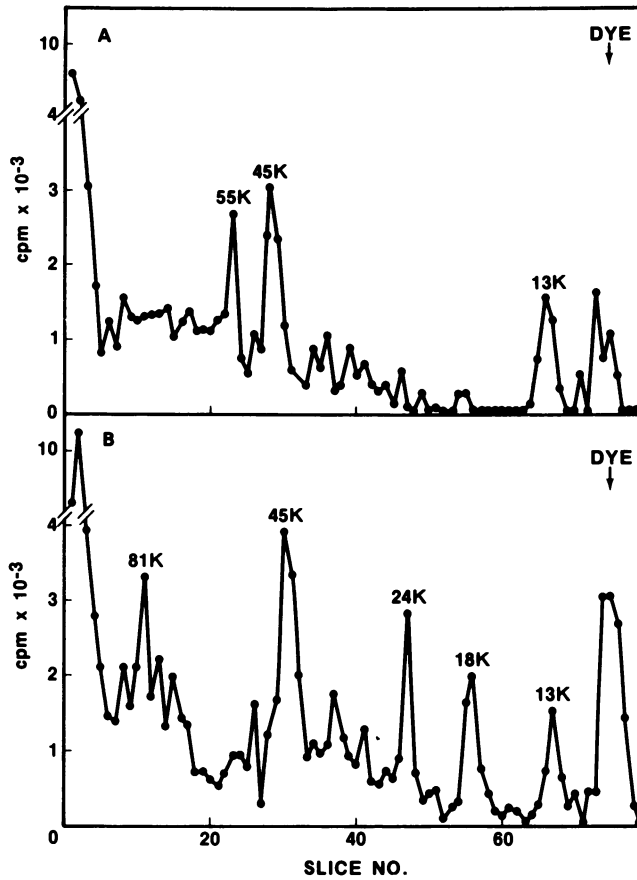

FIG. 1. *SDS-polyacrylamide gel electrophoresis of ^{125}I-labeled HTLV$_{CR}$. The intact (A) and SDS-disrupted (B) virus preparations were labeled with ^{125}I and chloramine-T and run in 12% SDS-polyacrylamide gels as described in the text.*

disrupted. This suggests that p24 and p18 are viral proteins present in the inner core of the virus. They appear to be analogous to the 24,000- to 30,000- and the 15,000-mol-wt core proteins of several retroviruses.

Purification and immunoprecipitation of p24. Our initial approach was to purify the p24 protein and develop immunological assays by using the purified protein. The procedure used was similar to methods used for other retroviral p30s. The HTLV$_{CR}$ was disrupted in 0.8 M NaCl and 0.5% Triton X-100, and the extract was freed of nucleic acids by passage through DEAE-cellulose at 0.3 M NaCl. The unadsorbed proteins were further chromatographed on phosphocellulose, the bound proteins being eluted with a linear salt gradient between 0 and 600 mM NaCl. The elution of the different proteins from the column was followed by analyzing 50-μl aliquots of the gradient fractions by electrophoresis in 12% polyacrylamide slab gels in the presence of SDS. The p24 eluted from the column as a very sharp peak between 150 and 250 mM NaCl. This is similar to the elution behavior of the 24,000- to 30,000-mol-wt proteins of other retroviruses during phosphocellulose column chromatography. The material was further purified by gel filtration on a Bio-Gel p60 column. Its profile in SDS-polyacrylamide gel electrophoresis after labeling with ^{125}I is shown in Fig. 2.

A rabbit antiserum prepared against detergent-disrupted HTLV$_{CR}$ precipitated HTLV$_{CR}$ ^{125}I-p24. Detergent-disrupted unlabeled HTLV$_{CR}$ competed fully with this precipitation at limiting antiserum levels (Fig. 3). However, none of several type C viruses (R-MuLV, FeLV, SSV, and BaEV) and type-D viruses (MPMV and SMRV) competed in the same assay. BLV, whose major internal structural protein has the same mol wt (24,000) as HTLV$_{CR}$ p24, and langur and macaque viruses also did not compete in the assay (data not shown).

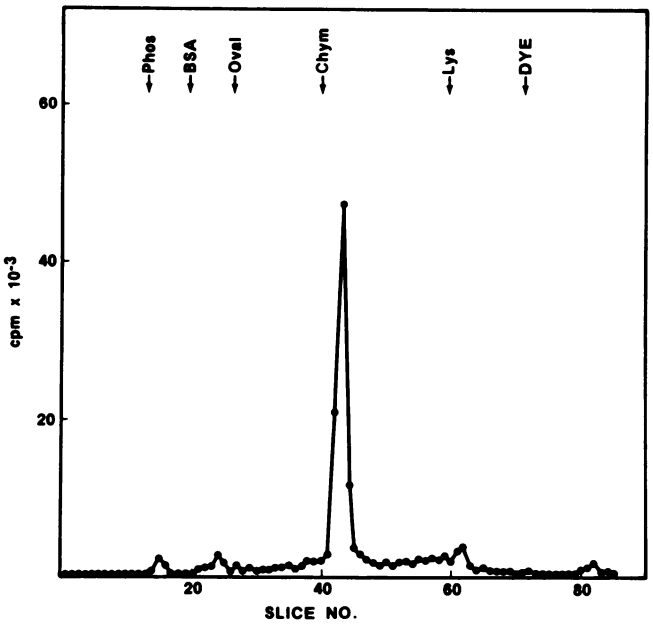

FIG. 2. *SDS-polyacrylamide gel electrophoretic profile of HTLV$_{CR}$ p24. The Bio-Gel fraction with maximum HTLV p24 was labeled with ^{125}I and run in 12% SDS-polyacrylamide gel. The mol wt standards run on a parallel gel were: phosphorylase b (Phos) (93,000); bovine serum albumin (BSA) (68,000); ovalbumin (Oval) (43,000); chymotrypsinogen (Chym) (25,500); and lysozyme (Lys) (14,000).*

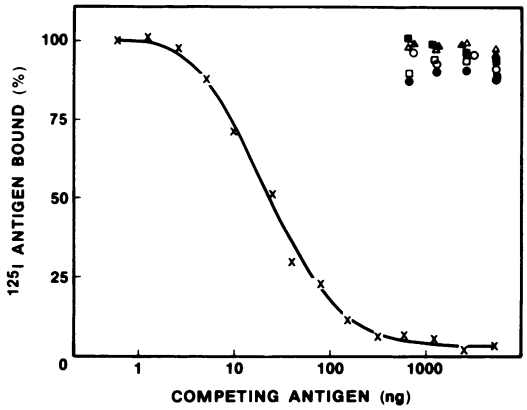

FIG. 3. *Homologous competition radioimmunoassay for HTLV$_{CR}$ p24. Triton X-100- and NaCl-disrupted mammalian retroviruses were tested for their ability to compete in the radioimmunoassay with ^{125}I-labeled HTLV$_{CR}$ p24 and limiting amounts of a rabbit antibody to HTLV$_{CR}$. Purified viruses used in the assay were HTLV$_{CR}$ grown in HUT102 cells (×), R-MuLV, (■), SSV (●), FeLV (□), BaEV (○), MPMV (△), and SMRV (▲).*

We also attempted to immunoprecipitate ^{125}I-labeled HTLV$_{CR}$ p24 with antibodies raised against other type C and type D viruses or their major internal core proteins. The antibodies raised against R-MuLV, FeLV, SSV, SSV p30, BaEV(M7) p30, MPMV p27, SMRV, and BLV p24 failed to precipitate labeled HTLV$_{CR}$ p24. In control experiments, all these sera precipitated the homologous p30 protein (Table 1).

Presence of p24 in the cores of HTLV$_{CR}$. With the availability of an immunological system to assay for p24 it was possible to screen different viral fractions for p24. It was particularly important to show that the p24 antigen we were studying was located in the core fraction of the virus, as expected from an analogy to p30's of other retroviruses. To this end, density-banded HTLV$_{CR}$ was treated in an ice bath for 5 min with 0.1% Sterox SL, a nonionic detergent that has been extremely useful in preparing core fractions from other retroviruses, and centrifuged to equilibrium through a 25 to 60% sucrose gradient. An untreated control sample was also run on a parallel gradient. The fractions collected from the gradients were assayed for reverse transcriptase activity to locate the cores. Aliquots from the same fractions were also assayed for p24 in a competition radioimmunoassay by using ^{125}I-labeled p24 and an antibody to disrupted HTLV$_{CR}$ as described in the legend to Fig. 3. As indicated in Fig. 4A, the detergent-treated sample had a distinct broad peak of core structures at their characteristic densities above 1.2 g/cm^3, whereas the untreated sample showed a sharp band at a density of 1.16 g/cm^3. It is

TABLE 1. *Comparison of the abilities of different antisera to bind ^{125}I-labeled HTLV$_{CR}$ p24*[a]

Antiserum to:	HTLV$_{CR}$ p24		Homologous p30	
	Maximum binding (%)	Titer[b]	Maximum binding (%)	Titer
HTLV$_{CR}$	74	12,000	74	12,000
R-MuLV p30	<10	<25	94	60,000
FeLV	<10	<25	96	867,000
SSV p27	<10	<25	97	120,000
BaEV(M7) p30	<10	<25	95	1,536,000
MPMV p27	<10	<25	87	153,600
SMRV	<10	<25	73	7,200
BLV p24	<20	<25	86	28,000

[a] Double antibody precipitations were done as described in the text.

[b] Titers are expressed as the reciprocal of the highest serum dilution capable of binding 20% of the appropriate ^{125}I-labeled protein.

important to note that these core fractions in the treated sample had significant amounts of p24 (Fig. 4B). It appeared that p24 was not as tightly bound in the nucleoprotein complex of the cores as the reverse transcriptase and some of it dissociated from the cores and appeared in the soluble fractions at the top of the gradient, but it was clear that p24 was a component of HTLV$_{CR}$ cores. The observation that p24 was labeled with ^{125}I only when HTLV was disrupted (Fig. 1) is consistent with it being a core protein.

Expression of HTLV$_{CR}$ p24 in human cells. To examine further the virus specificity of p24, competition radioimmunoassays were set up with ^{125}I-labeled p24 and the antibody to disrupted HTLV, and extracts of different cell lines were examined for their expression of HTLV$_{CR}$ p24. The cell lines tested included HUT102 cells (the cell line producing HTLV$_{CR}$), several other cutaneous T-cell lymphoma (CTCL) cells, acute lymphoblastic leukemia cells, normal human peripheral blood T-cells grown in the presence T-cell growth factor, human melanoma cells induced with iododeoxyuridine, and some human and primate B-cell lines containing or expressing Epstein-Barr virus. The only cells that were found positive for HTLV$_{CR}$ p24 were HUT102 cells and another CTCL line (CTCL-2) that also produces a virus very similar to or identical with HTLV$_{CR}$ (B. J. Poiesz, F. W. Ruscetti, M. S. Reitz, V. S. Kalyanaraman, and R. C. Gallo, submitted for publication) (Table 2). The HUT102 cell extract competed fully in the assay at about 10 μg of protein, whereas there was absolutely no competition with a human T-cell extract even at an input concentration of 400 μg of protein (Fig. 5). This result, along with data presented in Fig. 3, shows that p24 is unique to HTLV$_{CR}$ and to cells producing this virus.

Relation of HTLV$_{CR}$ to type C retroviruses. In view of the demonstrated relationship of other human virus isolates to some of the known animal retroviruses (8, 9), it is important to test the ability of HTLV$_{CR}$ to react in the competition radioimmunoassays of the structural proteins of other retroviruses. The most broadly reactive and well-studied protein of the known retroviruses is the major structural protein of mol wt 24,000 to 30,000. These proteins from SSV, BaEV, MPMV, and SMRV were purified by methods already described (5, 19; Markham et al., in press). Homologous and broadly reactive heterologous interspecies assays with the above purified proteins and antibodies to them were used to assay for competition by HTLV$_{CR}$. Since many of the putative human viruses reported earlier have turned out to be either indistinguishable from or closely related to the type C viruses of the SSV-GALV group and BaEV (7, 13, 16, 18), the competition radioimmunoassay profiles of these viruses were first examined. HTLV$_{CR}$ failed to compete in homologous assays for SSV p27 (Fig. 6A) and BaEV(M7) p30 (Fig. 6B). A broadly reactive radioimmunoassay with ^{125}I-labeled SSV p27 and antibody to FeLV was also used to compare HTLV$_{CR}$ to other mammalian type C viruses (1, 2). Many of the known type C viruses, e.g., SSV, FeLV, and BaEV(M7), competed fully in this assay (Fig. 6C). However, HTLV$_{CR}$ failed to compete in this assay.

Relation of HTLV$_{CR}$ to type D retroviruses. The precise classification of HTLV as a type C or type D virus is not clear. Its morphology has some features of both, but its mode of budding reflects more the pattern of a type C virus (16a). However, the higher mol wt (95,000) and the Mg^{2+} preference of reverse transcriptase (Rho et al., in press) suggested that the HTLV$_{CR}$ might be an atypical type C virus or one more closely related to the type D retroviruses. Therefore, we set up both homologous and heterologous competition radioimmunoassays with type D viral proteins to explore this possibility. HTLV$_{CR}$ failed to react in the homologous assays for MPMV p26 (Fig. 7A) and SMRV p36 (Fig. 7B), whereas MPMV and SMRV competed fully in their respective assays. An interspecies assay for the type D viruses has been described in the literature, in which all the three known type D viruses compete especially well with identical slopes. The competition has also been shown to be virus specific and not due to a primate-specific contaminant in the virus preparations (5). We tested the ability of HTLV$_{CR}$ to

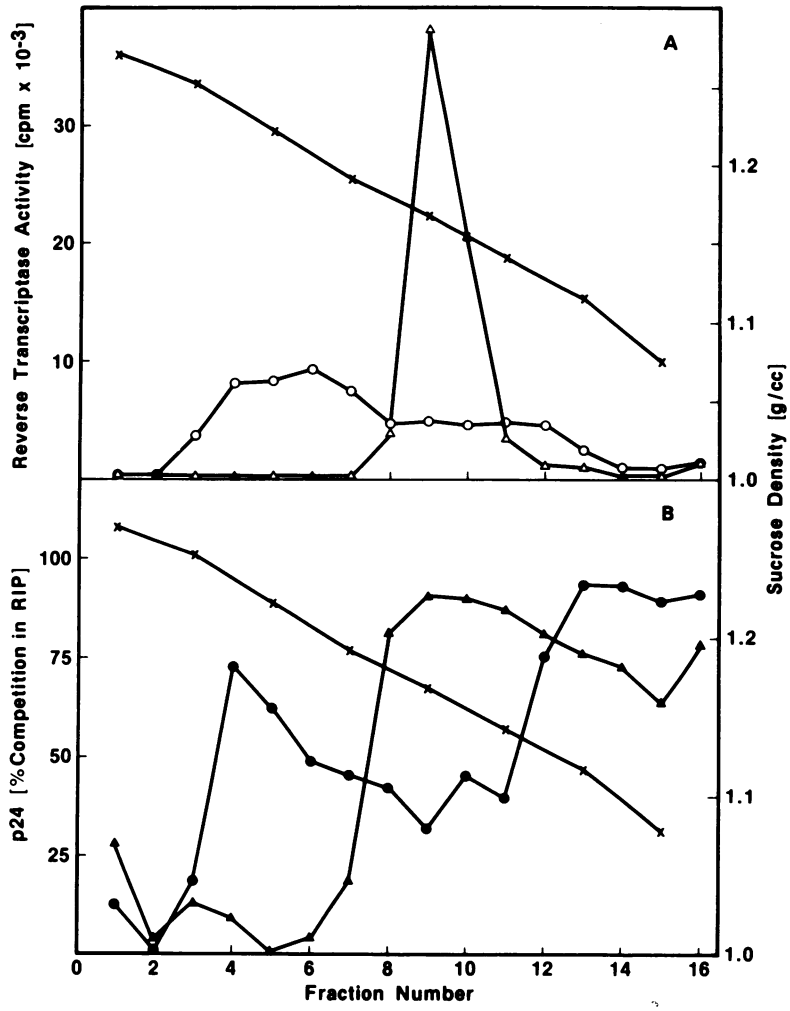

FIG. 4. *Density-banded HTLV was pelleted by centrifugation and resuspended in 10 mM Tris-hydrochloride (pH 7.5). A sample of 2 ml of virus suspension was treated with 0.1% Sterox SL (○, ●) for 5 min in ice and layered on top of a 9-ml 25 to 60% (wt/wt) sucrose gradient prepared in 10 mM Tris-hydrochloride (pH 7.5). An identical sample without Sterox SL (△, ▲) was layered on a parallel gradient. The gradients were centrifuged in a Spinco SW41 rotor at 35,000 rpm for 18 h. Fractions of 0.69 ml were collected from the bottom of the gradients, and samples were assayed for reverse transcriptase activity using (△, ○) (dT)$_{-15}$(A)n as primer template in the presence of Mg^{2+} as described elsewhere (16a). For p24 determinations (▲, ●), 2-μl samples were used in the competition radioimmunoassays as described in the legend to Fig. 3. Densities of the fractions (×) were determined by refractive index measurements with a Zeiss refractometer. Direction of centrifugation was to the left.*

compete in this interspecies assay using SMRV ^{125}I-p36 and goat anti-MPMV p27. As is evident from Fig. 7C, HTLV$_{CR}$ also did not compete in this assay, even at an input protein level of 5,000 ng, whereas complete competition was observed with SMRV and MPMV.

Relation of HTLV$_{CR}$ to type B and other unclassified retroviruses. HTLV$_{CR}$ did not compete in a group-specific assay for the major structural protein p28 of the type B virus, MMTV (Fig. 8A), utilizing ^{125}I-labeled p28 of C3HMTV and an antibody to *M. cervicolor* mammary tumor virus by the procedure of Teramoto and Schlom (20). BLV, like HTLV$_{CR}$, has a reverse transcriptase requiring Mg^{2+} and an internal strucutral protein of 24,000 mol wt (4). HTLV$_{CR}$ was therefore analyzed in the homologous radioimmunoassay for BLV p24. HTLV$_{CR}$

TABLE 2. *Screen of cultured human cells for HTLV p24 by competition radioimmunoassay*

Cell line	Competition with HTLV p24[a]
HUT102	+
CTCL-2	+
HUT78	−
Normal human T-cells[b]	−
T-9-44-p14[c]	−
ME-1[d]	−
P3HR-1 Cells	−
Daudi Cells	−
B-95 Cells	−
YT-4E[e]	−

[a] Homologous competition radioimmunoassay was done as described in the legend to Fig. 3. Cells were solubilized with 1 M NaCl and 0.5% Triton X-100 in 50 mM sodium phosphate buffer (pH 7.5) containing 0.5 mM phenylmethylsulfonyl fluoride and up to 200 μg of protein used in the assays. In the two samples that were positive, nearly complete competition was observed at 10 μg of protein.

[b] Grown in the presence of T-cell growth factor.

[c] Cultured human acute lymphoblastic leukemia cells.

[d] Human melanoma cells cultured with iododeoxyuridine.

[e] d Cultured Sezary cell line.

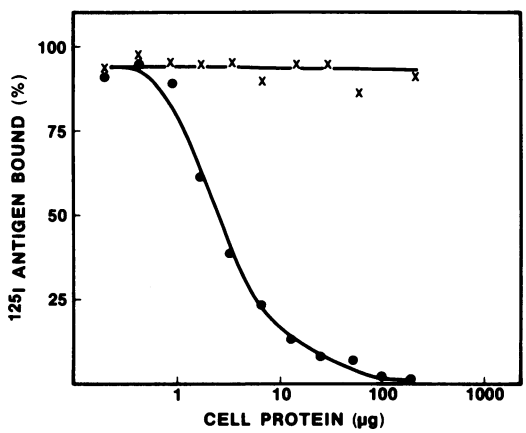

FIG. 5. *Expression of HTLV p24 in HTLV-producing cells. The homologous competition RIA was done as described in the legend to Fig. 3. The solubilized cell extracts of HUT102 cells (●) producing HTLV$_{CR}$ and solubilized proteins of normal human T-cells (×) were used in the assay as competing unlabeled antigens.*

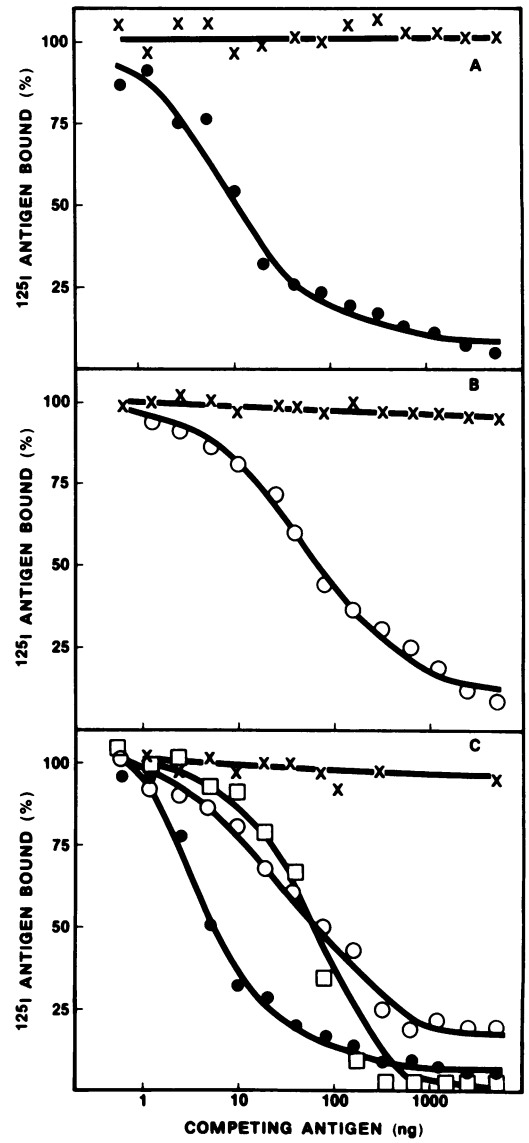

FIG. 6. *Competition radioimmunoassay for the major structural protein of type C viruses. (A) Homologous radioimmunoassay with ^{125}I-labeled SSV p30 and antibody to SSV p30. (B) Homologous radioimmunoassay with ^{125}I-labeled BaEV (M7) p30 and antibody to M7 p30. (C) Interspecies radioimmunoassay for type C viruses with ^{125}I-labeled SSV p30 and antibody to disrupted FeLV. Symbols: ●, SSV; □, FeLV; ○, BaEV; and ×, HTLV$_{CR}$.*

did not compete in this system, whereas 2 ng of BLV p24 gave 50% competition (Fig. 8B). HTLV$_{CR}$ was also tested for its ability to compete in the homologous radioimmunoassays for GPV p26, OMC-1 p30 and DKV p30 (Table 3), and the heterologous assays for OMC-1 p30 (with antibody to reticuloendotheliosis virus) and DKV p30 (with antibody to BALB-2 virus). HTLV$_{CR}$ did not compete in the above radioimmunoassays (Table 4). Also, HTLV$_{CR}$ did not compete in the homologous and interspecies assay for the major structural protein of VRV. The

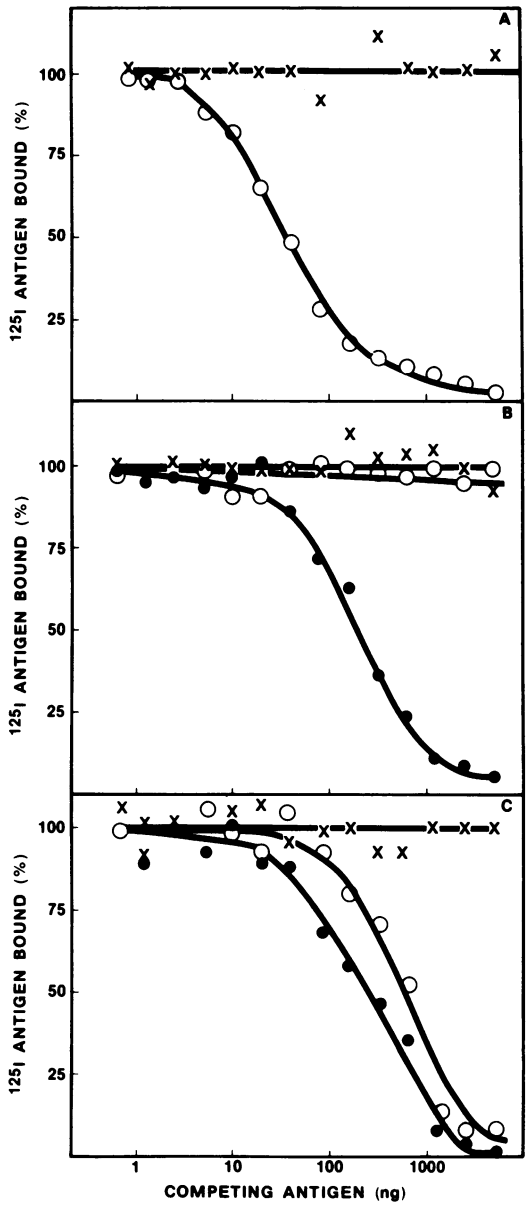

FIG. 7. *Competition radioimmunoassay for the major structural protein of type D retroviruses. (A) Homologous assay for MPMV p26 with antibody to MPMV and ^{125}I-labeled MPMV p26. (B) Homologous assay for SMRV p36 with ^{125}I-labeled SMRV p36 and antibody to SMRV. (C) Interspecies radioimmunoassay for type D viruses. ^{125}I-labeled SMRV p36 and antibody to MPMV p26 were employed. The viruses used in the assay were: MPMV* (○), *SMRV* (●), *and HTLV$_{CR}$* (×).

interspecies assays were done with labeled VRV p24 as the probe and limiting amounts of antibody to MPMV (1).

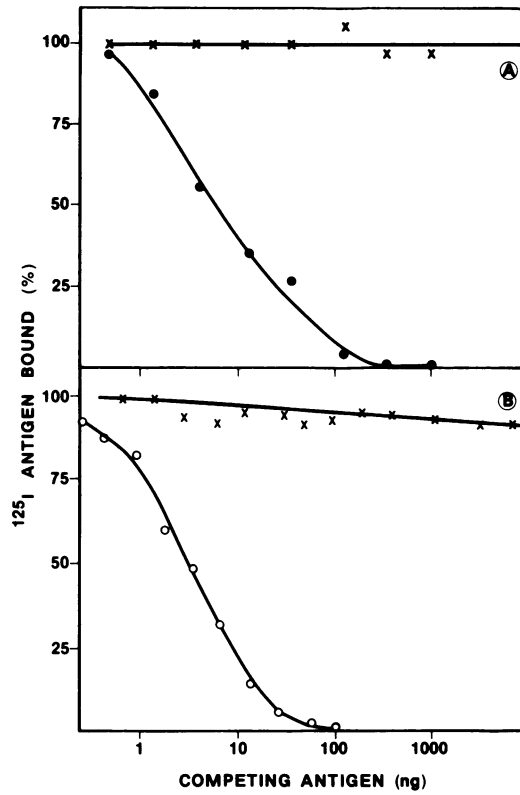

FIG. 8. *(A) Interspecies radioimmunoassay for MMTV p28. ^{125}I-labeled MMTV p28 and antibody to Mu·MTV were used in the assay. (B) Homologous competition radioimmunoassay for BLV p24. Competing viruses used were: MMTV* (●), *BLV p24* (○), *and HTLV$_{CR}$* (×).

DISCUSSION

The relationship of HTLV, a retrovirus isolated from a cultured human lymphoma cell line, to other retroviruses has been examined. Molecular hybridization studies indicated that HTLV$_{CR}$ is not significantly related to any of the known type B, type C, and type D viruses. The sequences related to HTLV$_{CR}$ are also not endogenous (genetically transmitted) to human cells (16b). On the other hand, retroviruses which are unrelated to one another by nucleic acid hybridization may still share antigenic determinants in their proteins which can be detected by appropriate radioimmunoassays (1). Thus, MPMV and SMRV, which share less than 3% of nucleic acid sequences, still share sequences in their major structural protein, which can be detected in the interspecies assay of the two viruses (Fig. 7C and reference 5). Similarly, macaque virus and BaEV share less than 2% nucleic acid homology and once again are antigenically related to each other (3). Hence, an

attempt was made to relate $HTLV_{CR}$ to the known retroviruses by immunological assays.

Enzyme activity neutralization studies using antibodies to reverse transcriptases showed no relatedness between the reverse transcriptase of $HTLV_{CR}$ and the enzymes of several type B, type C, and type D retroviruses (Rho et al., in press). The present study was designed to further analyze the relatedness of $HTLV_{CR}$ to other retroviruses using the immunological characteristics of the major internal protein of the virus (p24).

TABLE 3. *Comparison of the immunological relationship of $HTLV_{CR}$ with other retroviruses*

Competing virus	% Competition[a]
$HTLV_{CR}$	98
Type C viruses	
R-MuLV	0 (99)[b]
SSV	0 (100)
BaEV	5 (91)
FeLV	0 (100)
OMC-1	0 (95)
DKV	0 (85)
BLV	6 (98)
Type D viruses	
MPMV	0 (96)
SMRV	7 (96)
Type B viruses	
MMTV	0 (97)
Unclassified viruses	
GPV	8 (99)
VRV	0 (100)

[a] Homologous competition radioimmunoassays were carried out as described in the text using 10,000 cpm of the ^{125}I-labeled p24 of $HTLV_{CR}$ and limiting dilutions of the corresponding antiserum. The extent of competition obtained with 5 μg of the Triton X-100–NaCl-disrupted viruses is given.

[b] Numbers within parenthesis represent the percent competition exerted by the virus in the immunoprecipitation of its p30 by the homologous antibody.

The fact that p24 was radiolabeled with ^{125}I only when $HTLV_{CR}$ was solubilized suggests that the p24 was of viral origin and that it was an internal protein of $HTLV_{CR}$. The purification pattern of $HTLV_{CR}$ p24 also suggested that it was very similar to the major structural protein of type C and type D viruses. As expected, the isolated core structures were positive for p24. The expression of $HTLV_{CR}$ p24 was observed only in the $HTLV_{CR}$-producing cells, but not in a variety of cells including the virus-nonproducing cells of similar origin (Table 2). This result further indicates that p24 is a viral protein. None of the tested type C and type D viruses, including BLV, reacted in the assay. This supports previous results from nucleic acid hybridization (16b) and from studies of the antigenic relatedness of reverse transcriptase (Rho et al., in press), indicating that HTLV is novel.

$HTLV_{CR}$ did not compete in the homologous p30 radioimmunoassays for several of the known type B, type C, and type D viruses, and the unclassified BLV (Table 3). $HTLV_{CR}$ also did not cross-react in the interspecies assays of the type C and type D viruses (Table 4). Considering the fact that the 24,000- to 30,000-mol-wt internal virion core protein is a highly conserved interspecies determinant, all of these results indicate that $HTLV_{CR}$ is a novel retrovirus isolated from a human T-cell line. Nucleic acid hybridization studies have identified $HTLV_{CR}$-related sequences in the DNA of fresh neoplastic lymphoblasts from two patients; one a child with T-cell acute lymphatic leukemia, and the other a woman with the leukemic phase of Sezary syndrome (Poiesz et al., submitted for publication;

TABLE 4. *Analysis of interspecies antigenic determinants in $HTLV_{CR}$ structural proteins*[a]

Interspecies radioimmunoassays	Competing retroviruses								
	$HTLV_{CR}$	R-MuLV	FeLV	SSV	BaEV	MPMV	SMRV	VRV	MMTV
Anti-FeLV versus ^{125}I-R-MuLV p30	<5	100	100	96	83	<5	<5	ND[b]	<5
Anti-BaLB-2 virus serum versus ^{125}I-DKV p30[c]	<5	100	100	100	100	<10	<10	ND	ND
Anti-MPMV p27 versus ^{125}I-SMRV p36	<5	<10	<10	<10	<10	91	98	<10	10
Anti-MPMV versus ^{125}I-VRV p24[d]	<5	<5	<5	<5	<5	100	10	100	ND
Anti-Mc·MTV versus ^{125}I-MMTV p28	5	ND	ND	ND	ND	ND	ND	ND	100

[a] Interspecies radioimmunoassays were done as described in the text.

[b] ND, Not done.

[c] Values from reference 1.

[d] Values from reference 2.

M. S. Reitz, B. J. Poiesz, F. W. Ruscetti, and R. C. Gallo, *in* R. Neth and K. Mannweiler (ed.,) *Modern Trends in Human Leukemia IV*, in press). Further, retrovirus particles similar or identical to HTLV$_{CR}$ have recently been isolated from the cultured neoplastic T-lymphoblasts from this same Sezary patient (Poiesz et al., submitted for publication). The relationship of these viral particles to human T-cell neoplasias is being investigated.

ACKNOWLEDGMENTS

We thank S. A. Aaronson and M. Barbacid for the assistance in setting up the radioimmunoassays for GPV, VRV, and OMC-1 and for providing VRV proteins; J. Schlom for carrying out the radioimmunoassays for MMTV p28; G. Todaro for providing the macaque and langur viruses; and A. Burny for the BLV p24 and antibody to BLV p24. We acknowledge the excellent technical assistance of M. Jarvis.

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