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Characterization by Nucleic Acid Hybridization of HTLV, a Novel Retrovirus from Human Neoplastic T-Lymphocytes

M. S. Reitz, jr., B. J. Poiesz, F. W. Ruscetti, and R. C. Gallo

Recently this laboratory reported the isolation of a retrovirus from a patient with cutaneous T-cell lymphoma (mycosis fungoides) (Poiesz et al., to be published). Several different tissue specimens from this patient were positive for virus production, including malignant lymph node tissue and peripheral blood samples. In addition, a similar or identical virus was also isolated from a patient with cutaneous T-cell leukemia (Sezary leukemia) Gallo, see this volume). The virus displayed all the properties of a type-C retrovirus, including virus budding, viral type reverse transcriptase, and poly(A)-containing 70S RNA. We have prepared cDNA and 70S RNA from this virus, called HTLV, and used these as probes to determine: (1) the degree of relatedness to other viruses, (2) whether or not HTLV is a human endogenous virus, and (3) whether related sequences could be found in other human tissues.

HTLV cDNA hybridizes 90% to its own 70S RNA with a $Crt_{1/2}$ of 0.15, which is similar to the kinetics of hybridization of other type-C viral cDNAs to their homologous 70S RNAs. HTLV cDNA hybridizes to cytoplasmic RNA from HUT 102 (the T-cell line producing the first HTLV isolate) with a $Crt_{1/2}$ of 50–60, which is indicative of a viral RNA content of about 0.2% by weight. HTLV cDNA hybridizes to HUT 102 cell DNA with a $Cot_{1/2}$ of 900–1000 compared with a $Cot_{1/2}$ for cell unique DNA of 2200, indicating that the provirus is present at about 2–3 copies per haploid genome.

The HTLV cDNA does not hybridize significantly to 70S RNA of a wide variety of retroviruses, including murine and feline leukemia and sarcoma viruses, baboon endogenous virus, Mason-Pfizer virus, squirrel monkey retrovirus, bovine leukemia virus, RD 114 endogenous cat virus, murine mammary tumor virus, or avian myeloblastosis virus (Table 1). Very low levels of hybridization (5%–10%) are achieved with 70S from woolly monkey virus and gibbon ape leukemia virus. In reciprocal experiments no hybridization was ob-

Table 1. Lack of relatedness of HTLV to other retroviruses[a]

Viral 70S RNA from	% Hybridization of HTLV ^{3}H-cDNA
HTLV	90
SSV/SSAV	16
$GaLV_H$	13
MuLV	9
MuSV	9
FeLV	8
FeSV	7
BaEV	7
M-PMV	3
SMRV	6
BLV	11
MMTV	8
AMV	4
RD114	3

[a] HTLV ^{3}H-cDNA was hybridized to 1 μg of the indicated 70S viral RNA to a Crt ≥2, then assayed for hybridization by S1 nuclease digestion. Values are not normalized or corrected for t=0 values. AMV, avian myeloblastosis virus; SSV, simian sarcoma virus; SSAV, simian sarcoma associated virus; $GaLV_H$, gibbon ape leukemia virus, Hall's Island strain; MuLV, murine leukemia virus; MuSV, murine sarcoma virus; FeLV, feline leukemia virus; FeSV, Feline sarcoma virus; BaEV, baboon endogenous virus; M-PMV, Mason-Pfizer monkey virus; SMRV, squirrel monkey retrovirus; BLV, bovine leukemia virus; MMTV, murine mammary tumor virus

Table 2. Distribution of HTLV-related sequences in human DNA

DNA-from	No. samples tested	No. samples:		
		Negative	Intermediate	Positive
1. Cultured CTCL, lines[a]	3	1	0	2
2. Myelogenous leukemia lines	3	3	0	0
3. Fresh CTCL (Sezary) peripheral blood	2	1	0	1
4. Autopsy tissue, M. fungoides	1	1	0	0
5. Fresh peripheral blood, ALL	3	1	1	1
6. Fresh peripheral blood, CLL	6	6	0	0
7. Fresh peripheral blood, AML	5	5	0	0
8. Fresh peripheral blood, CML	7	7	0	0
9. Burkitt tumor	2	2	0	0
10. Normal autopsy tissue	10	9	1	0

[a] HTLV ^{3}H-cDNA was hybridized to 600 μg of DNA from the indicated tissues in 0.4 *M* NaCl (65°), then assayed by digestion with S1 nuclease. Negative indicates >20% of the homologous hybridization, intermediate indicates 20%–40% of the homologous hybridization. Positive samples gave 50%–80% of the homologous hybridization. One of the two positive CTCL lines is HUT 102. CTLC, continuous T-cell line; ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia

served to HUT 102 cytoplasmic RNA or cell DNA with cDNA from the above animal viruses, i.e., only cDNA from HTLV hybridized to the nucleic acids from the human T-cell lymphoma cell line. No hybridization was observed to the proviral DNA of a number of species harboring endogenous retroviruses, including langur, owl monkey, baboon, squirrel monkey, colobus, macaque, cat, rat, mouse, guinea pig, and hamster. Therefore, HTLV is not related to any of the endogenous retroviruses of these species.

Although HTLV hybridizes to DNA and cytoplasmic RNA from HUT 102, the infected HTLV-producing cell line, no hybridization is obtained with DNA or RNA from normal human peripheral blood T-lymphocytes, stimulated in short-term culture (72 h) with phytohemagglutinin (PHA) or with DNA from a number of non-neoplastic autopsy tissues or peripheral blood samples from patients with acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), or chronic lymphocytic leukemia (CLL) (Table 2). DNA from one out of three acute lymphocytic leukemias (ALL) and one out of two fresh (uncultured) Sezary syndrome peripheral blood samples hybridized a significant (20%–25% compared to 40% for the homologous DNA) amount of HTLV cDNA. The positive ALL was a T-cell ALL.

A T-cell line was established from the positive Sezary T-cell leukemia sample, and a second isolate of type-C virus was obtained (Poiesz et al., unpublished work). HTLV cDNA hybridizes to DNA and cytoplasmic RNA from the cell line (CTCL-2) producing the second virus to about the same extent as do the homologous DNA and RNA. The Tm of the hybrid formed with HTLV cDNA and CTCL-2 RNA is identical to the homologous. Virus from CTCL-2 was used to prepare cDNA. This cDNA hybridized to HTLV 70S RNA but not to AMV 70S RNA. The above data indicates that the virus from CTCL-2 is closely related to HTLV.

Detailed descriptions of these viruses and their isolation are presented elsewhere in this symposium (Gallo). It would appear from the data above that HTLV is a novel retrovirus isolate not related to previously described retroviruses to a significant extent and that it is not an endogenous human virus. It appears instead to be an acquired virus which has infected some humans, and the preliminary data suggests that it is specifically associated with T-cell lymphomas and leukemias of a mature cell type. Studies are underway to further explore the role of HTLV in these diseases.

Reference

Poiesz BJ, Ruscetti FW, Mier JW, Woods AM, Gallo RC (to be published) Proc Natl Acad Sci USA