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12. Eight log-phase plates of Chinese hamster ovary fibroblasts (CHO-K1) were extracted in 50 m*M* sodium phosphate buffer, *p*H 7.4. The extract was passed over a G-25 column equilibrated in buffer, and 50 μl of eluent was incubated with 6 m*M* $MgCl_2$, 300 μ*M* PRPP, and 500 μ*M* ZMP. The reaction was performed at 37°C for 60 minutes. Results were analyzed by anion-exchange high-performance liquid chromatography with the nucleotide buffer system described earlier (*3*).
13. Adenylate and guanylate kinase were purchased from Sigma Chemical Co., St. Louis, Mo. One unit of enzyme was incubated in 50 m*M* tris-HCl, *p*H 7.4, with 10 m*M* $MgCl_2$, 100 m*M* KCl, 5 m*M* ATP, and 100 μ*M* of AMP, GMP, or ZMP. Assays were performed at 37°C for 1 hour. Analyses were performed as described in (*12*). For explanation of abbreviations, see legend to Fig. 1.
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16. Purified enzyme was stored at −70°C at a concentration of 0.5 mg/ml in 50 m*M* sodium phosphate with 0.3 m*M* ATP, 6 m*M* $MgCl_2$, and 1 m*M* dithiothreitol (*p*H 7.4). For assay of the reverse reaction, purified enzyme was diluted 1:30 with buffer containing 70 m*M* tris-HCl, *p*H 7.4, plus 700 μ*M* EDTA, 1.7 m*M* β-mercaptoethanol, 7 m*M* $MgCl_2$, 33 m*M* potassium phosphate, and bovine serum albumin (1 mg/ml). All reactants were also dissolved in this buffer. All assays were performed at 37°C, and product formation was linearly related to time of incubation and amount of enzyme added for various times and at a fixed substrate concentration. When varying the ZMP concentration, 0.2 to 2 m*M*, PRPP concentration was fixed at 300 μ*M*. When varying the PRPP concentrations 10 to 300 μ*M*, ZMP concentration was fixed at 10 m*M*. For assay of the forward reaction, purified enzyme (diluted 1:60 with buffer) was incubated in the buffer described above. Ribose 5-phosphate was fixed at 300 μ*M* and the concentration of ATP was fixed at 300 μ*M* or the concentration of ZTP varied from 20 to 500 μ*M*, respectively.
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Novel Viral Sequences Related to Human T-Cell Leukemia Virus in T Cells of a Seropositive Baboon

Abstract. *Antibodies reactive with proteins of human T-cell leukemia virus (HTLV) can be found in Old World monkeys. A T-lymphocyte cell line established from a seropositive baboon (*Papio cynocephalus*) was analyzed for the presence of viral DNA sequences. The provirus found in these cells was related to but distinct from HTLV subgroup I. These results add to recent evidence from human studies that HTLV represents a spectrum of infectious T-lymphotropic retroviruses that includes closely and distantly related members.*

Human T-cell leukemia virus (HTLV) is a family of related T-cell tropic retroviruses associated with a specific subtype of mature T-cell malignancy in man (*1*). Two subgroups of HTLV have been identified thus far. Members of the first subgroup, HTLV-I, are highly related to each other, if not identical. These include the initial isolates of the virus in the United States (*2, 3*) as well as additional isolates from patients in this country (*4, 5*), Israel (*4*), South America (*4*), Japan (*4, 6*), and the Caribbean (*4*). Members of the second subgroup, HTLV-II, are only distantly related to HTLV-I as determined by serological cross-reactivities of their proteins (*7*) and molecular hybridization studies with cloned viral genomes (*8, 9*). Only two HTLV-II isolates have been obtained, the first from a patient with a T-cell variant of hairy cell leukemia, and the second from a patient with acquired immunodeficiency syndrome (AIDS) (*10*). Recently, antibodies against HTLV antigens have been found in several Old World monkey species, including Japanese and Chinese macaques, African green monkeys, and baboons (*11–13*). It has been proposed that HTLV can be spread between macaques and humans in Japan (*11*). However, we have evidence that macaques are also seropositive in regions of Japan where HTLV is not endemic in the human population (*13*), suggesting independent entries of the virus into man and other primates. We have analyzed DNA from a T-cell line established from a baboon that was seropositive for HTLV antigens. We found the viral sequences to be related to but distinct from HTLV-I and HTLV-II.

A T-lymphocyte cell line (991-ICC) was established from peripheral leukocytes of a baboon (*Papio cynocephalus*) that is seropositive for HTLV antigens by the enzyme-linked immunosorbent assay (ELISA). The baboon was a recipient of cellular material (bone marrow, lymph node, spleen, and peripheral blood) from a leukemic baboon from Sukumi, U.S.S.R., as part of a U.S.–U.S.S.R. joint virology study during 1973–1975 (*14*). The cell line releases typical type-C particles and extracellular reverse transcriptase, and reacts positively with a monoclonal antibody against the 19,000 dalton core protein (p19) of HTLV as well as a hyperimmune antibody against HTLV p24 (*15*).

High molecular weight DNA from 991-ICC was examined for the presence of HTLV-related sequences by Southern blot hybridization (Fig. 1). DNA from cell lines infected with HTLV-I (lane 1) and HTLV-II (lane 3) were used as positive controls and uninfected human cells were used as a negative control. DNA from many primates, including baboons, lacks sequences closely related to HTLV (data not shown). The restriction endonuclease Sst I cuts in the long terminal repeat (LTR) of many HTLV-I isolates and in the LTR, as well as internally in HTLV-II. When it was used to cut 991-ICC DNA a single band of 8.5 kb was produced that hybridized to the cloned HTLV-I genome. A similar size band was observed in the DNA of HTLV-I infected cells, corresponding to a complete genome with one LTR. This result indicates that like HTLV-I, the provirus in 991-ICC is only cut in the LTR by Sst I. However, Bam HI, which yields a characteristic internal band in all known HTLV-I isolates and two internal bands

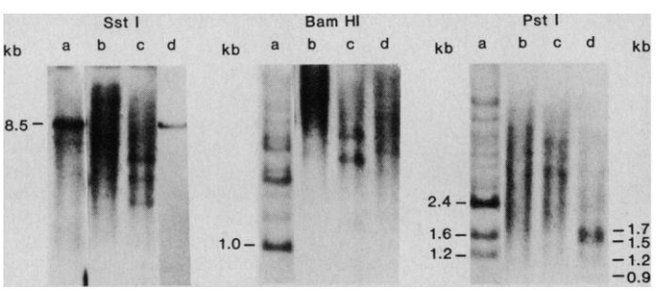

Fig. 1. The distinction between the provirus in 991-ICC and HTLV-I and HTLV-II. DNA from CH, an HTLV-I infected cell line (lane a), normal human thymus (lane b), MO, an HTLV-II infected cell line (lane c), and 991-ICC (lane d) were digested with the enzymes Sst I, Bam HI, and Pst I as shown, subjected to electrophoresis in 0.8 percent agarose gels, and transferred to nitrocellulose filters (*20*). Hybridization was carried out with ^{32}P-labeled DNA representing the complete HTLV-I genome that has been purified away from the vector on agarose gels. After incubation at 37°C for 20 hours in a solution containing triple-strength standard saline citrate, 50 percent formamide, 20 percent dextran sulfate, and 5× Denhardt's solution [0.1 percent bovine serum albumin, Ficoll, polyvinylpyrrolidone] and ^{32}P-labeled HTLV DNA (2×10^6 count/min), the filters were washed with two changes of double-strength SSC and 0.1 percent sodium dodecyl sulfate (SDS) at room temperature for 10 minutes each and at 65°C for another 10 minutes. Autoradiography was for 20 to 48 hours.

of 5.0 kb and 3.5 kb in HTLV-II, did not give discernible discrete bands in 991-ICC DNA, suggesting that Bam HI may cut not at all or once in the provirus of 991-ICC and that the infected cell population is polyclonal. Pst I, which gives three internal bands of 2.4, 1.6, and 1.3 kb with HTLV-I genomes, generated four detectable bands of 1.7, 1.5, 1.3, and 0.9 kb from 991-ICC DNA. The multiple high molecular weight bands are probably junction fragments. Thus these experiments indicate that the provirus in 991-ICC is distinct from HTLV-I and HTLV-II. Hybridization to an HTLV-II probe revealed specific bands in HTLV-I and HTLV-II DNA but not in 991-ICC (Fig. 2), indicating the baboon provirus is more related to HTLV-I than to HTLV-II.

To determine whether the provirus in 991-ICC is homologous to only a restricted portion of HTLV-I, we used the complete clone as well as subclones containing only *pol* or *env*-pX sequences of HTLV-I. pX is a region at the 3′ end of the HTLV genome that has open reading frames for several small peptides (*16*). With the Pst I digestion, we were able to detect strong hybridization using all three probes (Fig. 3A) and we can assign the 1.7-kb band in the *pol* region, the 1.5-kb band overlapping the *pol* and *env* regions, and the 0.9-kb band in the *env* region. The 1.2-kb band detected by the total HTLV-I genome is probably located in the *gag* region for which a subclone is not available. All the hybridizable bands withstand stringent washing conditions of 0.5× SSC (standard saline citrate) and 65°C (Fig. 3B), suggesting that they are closely related to HTLV-I over the entire genome. The relatively weak hybridization signal of 991-ICC DNA to the HTLV-I probe compared to the homologous control (see Fig. 1) may be due to infection of only a small percentage of the 991-ICC cells. In fact, immunofluorescence analysis of these cells with antibodies against HTLV antigens showed that less than 10 percent of the cells express viral antigens (*15*).

The provirus that we have identified in cultured T lymphocytes from a baboon, although related to HTLV-I, is clearly distinct from all known HTLV-I isolates.

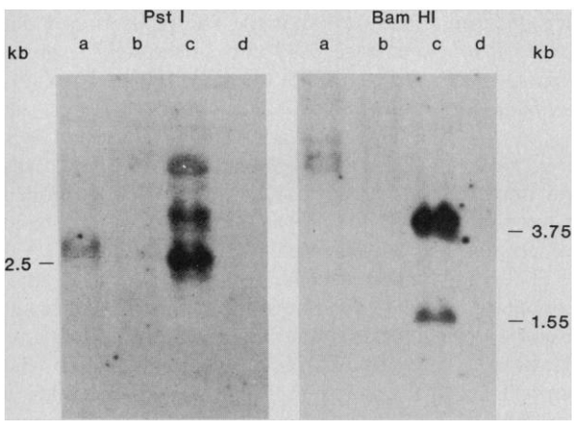

Fig. 2. Lack of detectable homology of 991-ICC provirus to HTLV-II. Duplicate filters, prepared as described in legend to Fig. 1, were hybridized to a subclone of HTLV-II corresponding to 2.5 kb of 3′ sequences. As in Fig. 1, lanes a to d are, respectively, CH (HTLV-I), normal thymus, MO (HTLV-II), and 991-ICC.

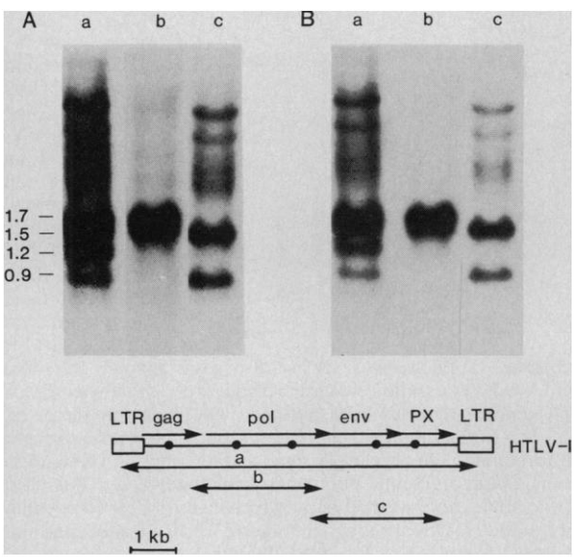

Fig. 3. Hybridization of specific HTLV-I probes to the 991-ICC provirus. 991-ICC DNA was digested with Pst I and blot hybridized to the entire HTLV-I genome (probe a), HTLV-I *pol* sequences (probe b), and *env*-pX sequences (probe c). The filters were washed in (A) double-strength SSC and 0.1 percent SDS at room temperature, and (B) 0.5× SSC and 0.1 percent SDS at 65°C.

Serological surveys have demonstrated clusters of HTLV carriers in southwestern Japan, the Caribbean basin, parts of South America, southeastern United States, and Africa. In the United States, healthy carriers of the virus are unusual and are found mainly in rural black populations. Although the survey of primates for a related virus has been less extensive, several Old World monkey species, including Japanese and Chinese macaques and African green monkeys and baboons, have been seropositive (*15*). In Japan, the distribution of virus in primates is much wider than in the human population (*15*). These observations suggest that the entry of virus into primates and man occurred independently and argues against a present-day transmission of virus between primates and man. Because of the widespread infection of Africans and because of the presence of Old World primates, including baboons, in Africa, we proposed that the origin of HTLV in the Caribbean, the United States, and South America was from entry of infected Africans to the Americas (*17*). Further, HTLV may have been brought to Japan by the 16th century Portuguese seamen who also had contact with Africa (*17*). Isolates of HTLV-I from patients in the United States, Japan, the Caribbean, and South America are closely related, if not identical, by restriction enzyme mapping (*8, 18*). Our present data suggest that the virus found in one baboon is related to but distinct from HTLV-I. Yamamoto *et al.* (*19*) have found that HTLV and a virus from an African green monkey contain identical core antigens but distinguishable envelope proteins. We also have evidence that more than one virus variant occurs in Old World monkeys (not shown), suggesting that infection of primates may have occurred much earlier than infection of man. Like HTLV, the primate viruses are T-cell tropic. We propose to name these viruses PTLV for primate T-lymphotropic viruses. Whether PTLV are involved in leukemogenesis in primates remains to be determined.

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A Transforming *ras* Gene in Tumorigenic Guinea Pig Cell Lines Initiated by Diverse Chemical Carcinogens

Abstract. *Fetal guinea pig cells were transformed by treatment with four different chemical carcinogens including nitroso compounds and polycyclic hydrocarbons. As a consequence of this treatment, oncogenes capable of transforming NIH/3T3 cells became activated in each of five independently established clonal guinea pig cell lines. Molecular characterization of representative NIH/3T3 transformants revealed that the same oncogene was present in each of the cell lines tested. Moreover, detection of this transforming gene paralleled the acquisition of tumorigenic properties by these neoplastic cells.*

Cells from various human tumors have oncogenes capable of inducing malignant transformation upon transfection into morphologically normal cells (*1–8*). Molecular characterization of these oncogenes revealed that in most cases they are members of the *ras* gene family (*8–12*). However, how these sequences control the expression of malignancy remains unknown. Transforming *ras* genes have also been detected in chemically induced tumors (*13–16*).

We conducted an investigation to determine whether chemically transformed guinea pig cells also contain transforming *ras* genes. This in vitro model system has several advantages for studying the role of oncogene activation in the multistep process of carcinogenesis (*17*). For instance, nontreated guinea pig cell cultures, unlike those of rat or mouse origin, do not transform spontaneously during continuous passage. Moreover, several distinct preneoplastic stages have been observed as carcinogen-exposed guinea pig cells develop into tumor cells (*17*). Expression of tumorigenicity in this model system is always preceded by the stages of morphologic alteration and morphologic transformation. Additional phenotypic properties, including anchorage-independent growth, are often expressed along with tumorigenicity. This indicates that multiple changes are necessary for the acquisition of the complete neoplastic phenotype (*17, 18*).

Chemically transformed guinea pig cell lines used in our studies were derived after exposure of fetal cells, either in vitro or in vivo, to four different chemical carcinogens (*17*). Cell line 74-C3 was derived by treatment in vitro of cells from 48-day fetal skin with 3-methylcholanthrene (3-MCA) and cloned in agar in its 64th subculture. Cell line 104-C1 was derived by exposure in vitro of cells from a 43-day-old fetus to benzo[*a*]pyrene and cloned in agar at its 20th subculture. Cell line 107-C3 was derived by treatment in vitro of cells from the same 43-day-old fetus that was used to generate 104-C1 cells—in this case the fetal cells were exposed to *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG), and the cell line was cloned in agar in its 50th subculture. Cell line HM4-C1 was derived from cells of a 49-day-old fetus that received a transplacental injection of MNNG 48 hours before removal from its mother—the cell line was cloned in agar at its 70th subculture. The HM2-C1 cell line was derived from cells of a 38-day-old fetus whose mother had received a single injection of diethylnitrosamine (DEN)—the cell line was cloned in its 85th subculture (*17*). As controls, a nontreated guinea pig fetal cell culture GP84 (*19*) and several other guinea pig fetal cell pools were used.

Approximately 20 μg of high molecular weight DNA extracted from each guinea pig cell line was precipitated with calcium phosphate and tested for the presence of transforming activity in transfection assays in which NIH/3T3 mouse fibroblasts were used as recipient cells (*20, 21*). DNA's isolated from each of the five chemically transformed cell lines induced foci of morphologically transformed cells after 2 weeks of incubation. Transformation efficiencies ranged from 0.08 to 0.15 foci per microgram of donor DNA, a frequency comparable to those obtained in our laboratory with DNA from T24 human bladder carcinoma cells (*6*). In contrast, no detectable morphologic alteration occurred in the NIH/3T3 cultures with DNA's isolated from six different cell pools derived from untreated embryos. These results indicate that an oncogene became activated in these transformed guinea pig cell lines as they progressed from the normal to the neoplastic state.

To demonstrate that transformation of NIH/3T3 cells was due to the uptake of

Table 1. Correlation between oncogene activation and development of malignancy in carcinogen-treated guinea pig fetal cells. Transformation efficiency is expressed as number of foci per microgram of DNA. Colony formation in agar was determined as follows: trypsinized single cells (10^2 to 10^5) were suspended in 4 ml of RPMI 1640 medium containing 10 percent fetal bovine serum and 0.35 percent agar and poured onto a solidified basal layer of 4 ml of RPMI 1640 medium but containing 0.5 percent agar, in 60-mm petri dishes. Colonies consisting of more than 100 cells were scored after 10 days of culture at 37°C and 8 percent CO_2 atmosphere. Results given are means of two experiments. Tumorigenicity data were obtained from (*17*).

Mass cultures and passage number	Clonal cells	Transformation efficiency	Colony formation in agar (%)	Tumorigenicity
107-P17		0	<0.001	−
107-P40		0	<0.001	−
107-P61		0.01	1.0	+
	107-C3	0.10	4.8	+
HM2-P33		0	<0.001	−
HM2-P67		0	<0.001	−
HM2-P86		0.05	1.1	+
	HM2-C1	0.08	1.4	+
HM4-P31		0	<0.001	−
HM4-P58		0	<0.001	−
HM4-P77		0.02	0.8	+
	HM4-C1	0.09	2.0	+