Proc. Natl. Acad. Sci. USA
Vol. 74, No. 1, 353–357, January 1977
Medical Sciences

Rauscher-leukemia-virus-related sequences in human DNA: Presence in some tissues of some patients with hematopoietic neoplasias and absence in DNA from other tissues

(human leukemia/multiple myeloma/Hodgkin's disease/nucleic acid hybridization/type-C RNA tumor virus)

GURMIT S. AULAKH* AND ROBERT C. GALLO†

Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20014

Communicated by Donald A. Glaser, October 12, 1976

ABSTRACT **A [^{3}H]cDNA probe synthesized from the RNA genome of Rauscher murine leukemia virus ($MuLV_R$) and purified by hybridization to $MuLV_R$ 70S RNA was hybridized to DNA from human normal and hematopoietic neoplasia tissues. This cDNA hybridized completely to its homologous 70S RNA and was free of self-complementary sequences. Sequences complementary to $MuLV_R$ cDNA were found in DNA from tissues of some patients with leukemia (2 of 8), Hodgkin's disease (3 of 10), and one patient with multiple myeloma. DNA from spleen and kidney of a patient with nonneoplastic disease did not contain detectable $MuLV_R$-related sequences. These virus-related sequences in the DNA from these neoplastic tissues were related but not identical to $MuLV_R$ sequences because differences of approximately 6° in the midpoints of thermal elution profiles were found between the heterologous and homologous duplexes. These nucleotide sequences are not the same as the proviral sequences of baboon type-C virus previously found from some other patients with leukemia [Reitz *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 73, 2113–2117; Wong-Staal *et al.* (1976) *Nature* 262, 190–195], because there is no sequence homology between nucleic acids from $MuLV_R$ and baboon virus. The absence of these nucleic acid sequences in many tissues of patients with neoplasia and from the few tissues examined from people with nonneoplastic disease suggests that they are not endogenous elements but are acquired after fertilization. Taken together with the previous detection of baboon and woolly monkey type-C viral related components in some human tumors, the results suggest acquisition of at least three types of type-C viral sequences in the human population.**

Data are accumulating that indicate the presence of type-C-tumor-virus-like components in some human tissues (1–23). Cytoplasmic nucleic acids with sequences related to the RNA of Rauscher murine leukemia virus ($MuLV_R$) and other viruses have been described (4, 9–17). Recently, nucleic acids and proteins related to the two known primate type-C virus groups—the simian sarcoma virus/gibbon ape leukemia virus group and the baboon endogenous virus group—have been reported in fresh human tissues (5–8, 11–14, 16, 18–23), although the reports of antigens related to p30 structural proteins (20–23) have been contested (24). Although clearly a rare event, isolation of a replicating virus from human tissues related to one or both of these primate type-C viruses has also been reported (25–30). Until recently, an important requisite for the demonstration of acquired type-C viral information in man, namely a DNA provirus in fresh human tissues, had not been met. Therefore, an extensive survey for proviral sequences in human DNA was initiated, using both labeled cDNA and 70S RNA probes from several RNA tumor viruses. Utilizing nonrecycled tritium-labeled cDNA probes or ^{125}I-labeled 70S viral RNA, initial work failed to reveal a DNA provirus related to the genome of $MuLV_R$, simian sarcoma virus, or gibbon ape leukemia virus in DNA of any human tissues (14). However, using ^{125}I-labeled 70S RNA of baboon endogenous type-C virus, recent experiments have shown nucleotide sequences of this virus in the DNA in a fraction of leukemic patients (16, 18). These findings indicate acquisition of baboon virus sequences or closely related sequences in these people. Lower amounts of *distantly related* ($\Delta\ t_e50$ of 6–9°, t_e being the midpoint of the thermal elution profile) nucleotide sequences were found in DNA of all human tissues examined (normal or tumor) (18, 31). Detection of the baboon virus nucleic acid sequences, however, does not explain the finding of cytoplasmic sequences related to the RNA of $MuLV_R$ and simian sarcoma virus.

The objective of experiments reported here was to examine DNA from human tissues for sequences related to RNA of $MuLV_R$ in more detail and with more carefully prepared cDNA probes. We report: (*i*) detection of nucleic acid sequences distantly related to $MuLV_R$ in DNA from spleens of some patients with acute myelogenous leukemia (AML), Hodgkin's disease, and multiple myeloma; (*ii*) absence of these sequences in DNA from apparently uninvolved tissues of the same patients with neoplasia and from the normal human tissues tested. Since $MuLV_R$ and the baboon endogenous virus are not detectably related (32), the detection of $MuLV_R$-related DNA sequences is not explained by the previously reported presence of proviral sequences of baboon virus in DNA from some leukemia patients. Taken together, the results show at least two independent sets of oncornavirus-related sequences in the DNA from some leukemia patients. While the mode, source, and time of acquisition, and relevance to etiology, must be speculative, interspecies transmission of one or both agents may be involved.

MATERIALS AND METHODS

Purification of DNA from Human Tissues. The tissues used in these experiments were uncultured specimens obtained at the time of autopsy and kept frozen at −80° until the isolation of DNA. The organ tissues obtained from people with neoplasia were infiltrated with tumor cells. The tissues were lysed in a

Abbreviations: $MuLV_R$, Rauscher murine leukemia virus; $NaDodSO_4$, sodium dodecyl sulfate; PB, phosphate buffer containing equimolar concentrations of monobasic and dibasic sodium phosphate at pH 6.8; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; AML, acute myelogenous leukemia; cDNA, DNA synthesized *in vitro* by detergent-disrupted virions of $MuLV_R$ and shown to be complementary to $MuLV_R$ 70S RNA; t_e50, midpoint of thermal elution profile; EC_0t, the productof time (seconds) and the DNA concentration (moles of nucleotides per liter) corrected for salt concentration. This was in practice calculated by multiplying ½ the absorbance at 260 nm of DNA samples by the time of hybridization in hours and then correcting for salt concentration of 0.48 M PB by multiplying by 5.6.

* Present address: Division of Virology, Bureau of Biologics, 8800 Rockville Pike, Building 29A, Room 2D-24, Bethesda, Md. 20014.
† To whom reprint requests should be addressed.

Table 1. Extent of hybridization of $MuLV_R$ cDNA to excess unlabeled cellular DNA from different tissues of patients with leukemia

Source of unlabeled cellular DNA			
Patient no.	Diagnosis	Tissue	Percent hybridization
1	AML	Spleen	15.8
		Kidney	2.2
		Liver	1.6
2	AML	Spleen	4.0
3	AML	Leukocytes	2.3
4	CML	Lung	42.8
		Liver	10.1
		Spleen	3.9
5	CML	Spleen	3.2
6	CML	Spleen	2.1
7	CLL	Leukocytes	1.9
8	CLL	Leukocytes	2.0
Controls	cDNA alone		2.0
	cDNA + $MuLV_R$ 70S RNA		94.0
	cDNA + DNA from $MuLV_R$-infected cells		86.0

As detailed in the *Materials and Methods* the hybridizations were carried out up to an EC_0t of 10,000. Background "sticking" of 2.0% of cDNA to the hydroxylapatite column was not subtracted. CML is chronic myelogenous leukemia; CLL, chronic lymphocytic leukemia.

solution containing 8 M urea, 1% sodium dodecyl sulfate ($NaDodSO_4$), 1 mM EDTA, 1 M $NaClO_4$, and 0.24 M NaH_2PO_4/Na_2HPO_4, pH 6.8 (PB). After extraction with chloroform, the DNA from these tissues was purified on hydroxylapatite as described by Britten *et al.* (33), treated with protease K, and sheared to 300 to 400 nucleotide pieces under pressure of 50,000 pounds/inch^2 (340 MPa). After dialysis the sheared DNA was concentrated by lyophilization. The 260 nm/280 nm absorbance ratio was between 1.90 and 1.97.

Preparation of $MuLV_R$ cDNA. $MuLV_R$ (5 ml) was layered over 30% (vol/vol) glycerol in TNE buffer (0.01 M Tris·HCl, pH 8.3; 0.15 M NaCl; 1 mM EDTA) and centrifuged at 100,000 × *g* for 1 hr at 4°. The resulting viral pellet was suspended in 0.15 ml of 0.01 M Tris·HCl buffer at pH 8.3. Nonidet P-40 (Shell Chemical Co.) was added to the final concentration of 0.005% and the mixture was incubated at 0° for 12 min. [^{3}H]-cDNA was synthesized in a reaction (final volume 2 ml) containing 0.1 M dithiothreitol; 100 μmol of NaCl; 12 μmol $MgCl_2$; 1 μmol each of [^{3}H]dATP (10 Ci/mmol), [^{3}H]dCTP (23 Ci/mmol), [^{3}H]dGTP (14 Ci/mmol), [^{3}H]dP (50 Ci/mmol); and 200 μg of actinomycin D. The mixture was incubated at 37° for 90 min; then NaCl and $NaDodSO_4$ were added to concentrations of 0.4 M and 1%, respectively. The mixture was deproteinized with TNE and Tris·HCl, pH 8.3, buffer-saturated phenol (5 min), then centrifuged at 5000 × *g* for 5 min. The cDNA was purified by Sephadex column chromatography and precipitated with 2 volumes of ethanol. After alkaline hydrolysis (0.3 M NaOH, 18 hr, 37°) the cDNA was recycled with poly(A) (0.4% $NaDodSO_4$ and 3 mM EDTA) and approximately 98% of the cDNA was recovered as single-stranded molecules. The single-stranded cDNA was then annealed to 70S $MuLV_R$ RNA (5 μg/ml) with an approximate equimolar ratio of RNA to cDNA by incubating for 20 hr at 66° (34, 35). After alkaline hydrolysis this purified single-stranded cDNA complementary to 70S viral RNA was used as a probe for DNA·DNA hybridization. The recovery after this step was 20% of the total single-stranded cDNA. The cDNA recycled with purified 70S viral RNA represents a more uniform transcript of the viral genome.

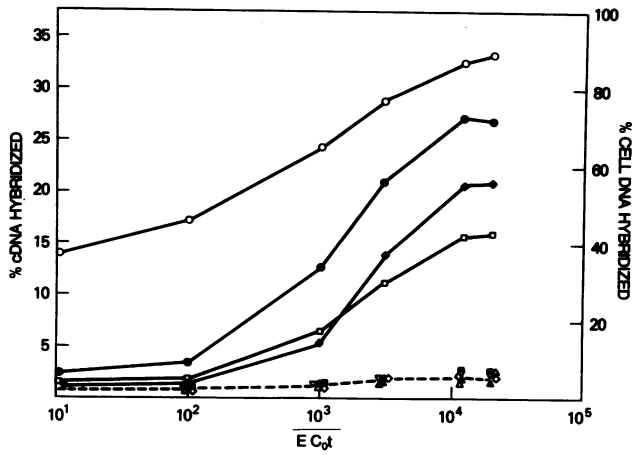

FIG. 1. Kinetics of reassociation of $MuLV_R$ cDNA and DNA from several different fresh tissues. [^{3}H]cDNA was hybridized to excess unlabeled tissue DNA as described in *Materials and Methods*. The % hybridization was determined for EC_0t values of 10–20,000. [^{3}H]cDNA reannealed with DNA from multiple myeloma spleen, ●—●; multiple myeloma kidney, △ - - △; aortic insufficiency spleen, ◇ - - ◇; aortic insufficiency kidney, ▽ - - ▽; AML spleen, □—□; AML liver, ▲ - - ▲; AML kidney, ■ - - ■; Hodgkin's disease spleen, ◆—◆; A_{260} of unlabeled tissue DNA, ○—○.

Molecular Hybridization. The hybridization mixture contained 5 mg/ml of unlabeled cell DNA and 45,000 cpm/ml of cDNA (0.75 ng) in a final volume of 100–300 μl of buffer of 0.48 M PB, 3 mM EDTA, and 0.4% $NaDodSO_4$. After denaturing at 105° for 5 min the mixture was incubated at 66° to EC_0t values (DNA concentration × time, corrected for salt concentration) which were varied from 10 to 20,000 (34). The hybrids were processed on a hydroxylapatite column pre-equilibrated at 60° with 0.14 M PB containing 0.4% $NaDodSO_4$. Radioactive material not adsorbing to hydroxylapatite under these conditions was considered to represent unhybridized single-stranded DNA. Hybridized DNA was then eluted with 0.48 M PB containing 0.4% $NaDodSO_4$. For determination of t_e50, the column was loaded as described and washed thoroughly with 0.14 M PB at increasing 5° increments up to 100°. The radioactivity was measured by adding 12 ml of Aquasol to 4 ml of eluate. Reannealing of unlabeled DNA was monitored by measuring absorbance at 260 nm.

RESULTS

Characteristics of the cDNA Probe. Self-complementary sequences were removed by self-annealing and nonhybridizable sequences, by prehybridization to viral RNA. This $MuLV_R$ cDNA hybridized 94% to homologous 70S $MuLV_R$ RNA and 86% to DNA of cells infected by $MuLV_R$ (Table 1). In the absence of viral RNA, 2% of the cDNA absorbed to the hydroxylapatite, representing nonspecific "background."

$MuLV_R$-Related Sequences in the DNA from Tissues of Leukemic Patients. $MuLV_R$ cDNA hybridized 15.8% to DNA from the spleen of patient 1, who had AML. No homology was found with DNA from the kidney or liver (Table 1). Reassociation (Fig. 1) reached a maximum at an EC_0t of 10,000. The $EC_0t_{1/2}$ suggests about one copy of the virus-related sequences per cell. The t_e50 of the duplexes was 74–75°, compared to a value of 81° for hybrids formed between this cDNA and $MuLV_R$ RNA (Fig. 2). Under identical conditions duplexes formed between the same $MuLV_R$ cDNA and DNA from cells

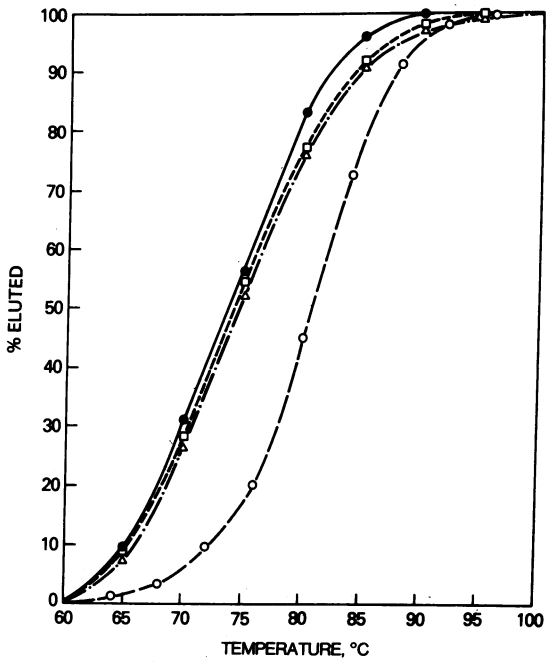

FIG. 2. Thermal stabilities of duplexes formed between $MuLV_R$ cDNA and DNA from different tumor tissues. At an EC_0t of 10,000 an aliquot was taken from the reaction mixture and applied to a hydroxylapatite column at 60° as outlined in *Materials and Methods*. The column was washed with 0.14 M PB containing 0.4% $NaDodSO_4$, and then the temperature was raised in 5° steps to 100° and the column was washed at each step. The % cDNA that eluted at each step was calculated, and the sum that eluted at a given temperature was plotted as a function of temperature. cDNA was also hybridized to 0.5 μg of homologous 70S $MuLV_R$ RNA for 12 hr. The hybrids were processed as above except the column was washed at 4° intervals. Labeled cDNA reannealed with: $MuLV_R$ 70S RNA, O — — O; multiple myeloma spleen DNA, □ - - □; AML spleen DNA, ●——●; and Hodgkin's disease spleen DNA, △ — · — △.

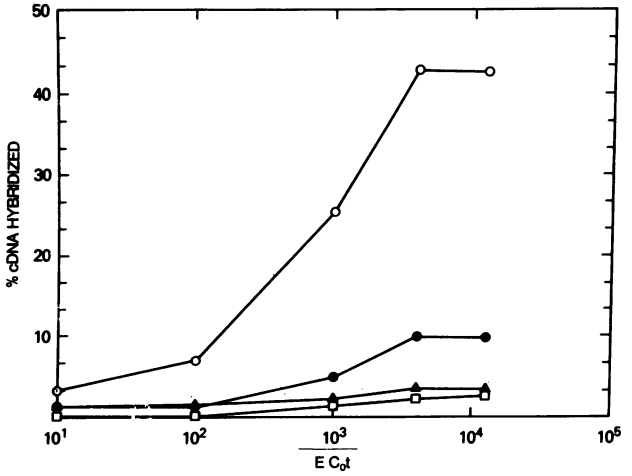

FIG. 3. Kinetics of reassociation of $MuLV_R$ cDNA and DNA from different tissues of a patient with CML. For details see *Materials and Methods*. The % hybridization was determined for EC_0t values of 10–20,000. cDNA reannealed with unlabeled: CML lung DNA, O—O; CML liver DNA, ●—●; CML spleen DNA, ▲—▲; and without cell DNA □—□.

infected by $MuLV_R$ also have t_e50 values of approximately 81°. Therefore, the low t_e50 of duplexes formed between $MuLV_R$ cDNA and DNA of some human tumor tissues shows that these sequences are not identical but distantly related to $MuLV_R$ sequences. These sequences were absent in DNA from two other patients with AML (nos. 2 and 3, Table 1).

In one patient with CML (patient 4), 42.8% of the cDNA hybridized to DNA from the lung (which was heavily infiltrated with leukemic cells), 10.1% to DNA from liver, and 3.9% to spleen DNA (Table 1). Reassociation kinetics shows that there is about one copy of the virus-related sequences per cell (Fig. 3). The thermal elution pattern (Fig. 4) shows a t_e50 of 78° (lung) and 75° (liver). Since the lung and liver were positive, the absence of $MuLV_R$-related sequences in the DNA from the spleen of this patient was surprising, especially because $MuLV_R$-related sequences were found in DNA from spleens of some patients with AML (Table 1), multiple myeloma, and Hodgkin's disease (Table 2). However, it could be due to the absence of tumor cells in the portion of the spleen examined, since the histology revealed only scattered leukemic cell infiltrates. $MuLV_R$-related sequences were absent in DNA from leukocytes of patients 7 and 8 with CLL (Table 1).

$MuLV_R$-Related Sequences in the DNA from Tissues of Patients with Solid Tumors. $MuLV_R$ cDNA hybridized 27.3% to DNA from spleen of a patient with multiple myeloma, but there was no detectable hybridization to the DNA from the kidney (Table 2). Again, the kinetics of reassociation (Fig. 1) indicate about one copy of virus-related sequence per cell, and the low t_e50 (74°–75°) indicates that the sequences are related, not identical, to $MuLV_R$ sequences.

DNA samples from the spleens of 10 patients with Hodgkin's disease were also examined. Three (nos. 10, 11, and 12, Table 2) were clearly positive (12.5, 20.5, and 11.3% hybridization, respectively); five (nos. 13–17) were questionable (4.5–6.7%) and two (nos. 18 and 19) were negative. The kinetics of reassociation, (Fig. 1) again show about one copy per cell of the virus-related sequences. The t_e50 was 74–75° (Fig. 2).

Absence of $MuLV_R$-Related Sequences in DNA from Tissues of a "Normal" Person. There was no hybridization to DNA from spleen and kidney of a patient with aortic insufficiency or to the DNA from the "normal" lung (not infiltrated

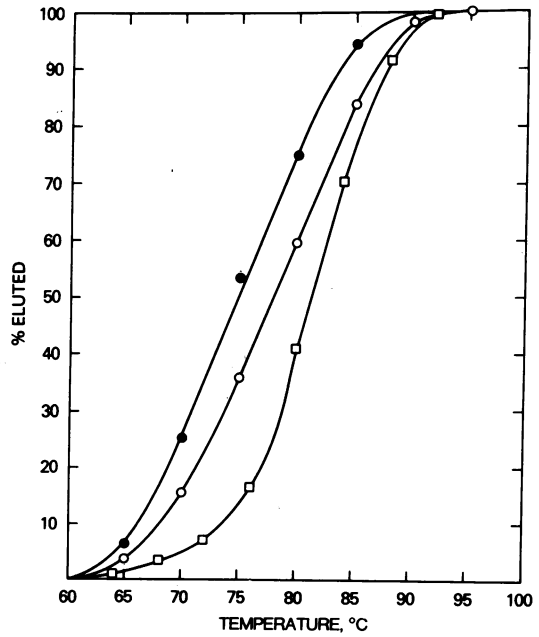

FIG. 4. Thermal stabilities of duplexes formed between $MuLV_R$ cDNA and DNA from tissues of a patient with CML. For details see the *legend* to Fig. 2. cDNA reannealed with: $MuLV_R$ 70S RNA, □—□; CML lung DNA, O—O; and CML liver DNA, ●—●.

Table 2. Extent of hybridization of $MuLV_R$ cDNA to excess unlabeled cellular DNA from tissues of patients with solid tumors

Source of unlabeled cellular DNA			
Patient no.	Diagnosis	Tissue	Percent hybridization
9	Multiple myeloma	Spleen	27.3
		Kidney	2.0
10	Hodgkin's disease	Spleen	12.5
11	Hodgkin's disease	Spleen	20.5
12	Hodgkin's disease	Spleen	11.3
13	Hodgkin's disease	Spleen	6.1
14	Hodgkin's disease	Spleen	6.7
15	Hodgkin's disease	Spleen	5.2
16	Hodgkin's disease	Spleen	4.5
17	Hodgkin's disease	Spleen	5.4
18	Hodgkin's disease	Spleen	2.5
19	Hodgkin's disease	Spleen	2.5
20	Aortic insufficiency	Spleen	2.9
		Kidney	2.5
21	Burkitt's lymphoma	Lung	4.3

See *Materials and Methods* and *legend* of Table 1 for details.

with tumor cells) of a patient with Burkitt's lymphoma (Table 2). Previous studies with normal leukocytes, including phytohemagglutinin-stimulated blood lymphocytes from many donors, also failed to reveal $MuLV_R$-related sequences in DNA of normal tissues (14).

Assay of Test DNA Samples for Contamination with Mouse Cellular DNA. Two DNA samples from tissues of patients 10 and 11 (Table 2) were tested by S. Spiegelman and R. Sweet for contamination with DNA from mouse and other rodents or with virus RNA. The mouse DNA contamination was ruled out because mouse mammary tumor virus cDNA did not hybridize to the human DNA. Contamination with other rodent DNA was examined by hybridizing the human tumor DNA with nonrepeated rat [^{3}H]DNA; no hybridization was found. The tumor DNA hybridized to $MuLV_R$ cDNA to the same degree before and after alkaline hydrolysis. This eliminates the interpretation that the data might be explained by contamination with viral RNA. Moreover, Spiegelman and Sweet have repeated the hybridization of these two human DNAs with other $MuLV_R$ [^{3}H]cDNA preparations and have obtained similar results (personal communication).

DISCUSSION

Nucleotide sequences related to $MuLV_R$ have been found in certain human neoplastic diseases. In one group of reports, Hehlmann *et al.* (9) and Kufe *et al.* (10) detected $MuLV_R$-related RNA in the cytoplasm of leukemic cells. Larsen *et al.* reported detection of Moloney-murine-sarcoma-virus-related sequences in the poly(A)-rich RNA of 22 of 47 leukemic patients (17), although Gallo *et al.* noted that similar sequences may be present in RNA from lymphocytes stimulated with phytohemagglutinin obtained from pooled blood of normal donors (14). Second, in a survey utilizing cDNA from reverse transcriptase (RNA-dependent DNA polymerase) reactions from cytoplasmic particles from fresh human hematopoietic neoplastic tissues, Spiegelman and colleagues found a portion of these sequences hybridized to $MuLV_R$ RNA (4, 9, 10). Similar findings were reported by Gallo *et al.* (11), Miller *et al.* (12), Mak *et al.* (13), and Reitz *et al.* (16) with cytoplasmic particles from fresh leukemic blood or bone marrow cells. The latter four reports also showed more sequence homology with RNA from simian sarcoma virus, and a recent study of tissues from one patient (HL-23) showed extensive homology of this cDNA with RNA from baboon endogenous virus (16). The sequences that hybridize to simian sarcoma virus RNA are not the same as those that hybridize to baboon virus RNA, because hybridization to a mixture of RNA from both viruses gave additive results (16). Third, utilizing again the cDNA prepared from an endogenous reverse transcriptase reaction of cytoplasmic particles from fresh human leukemic cells, Baxt and Spiegelman found that a portion of these sequences did not hybridize to DNA of normal human tissues but did hybridize to DNA from leukemic cells (4). Baxt later reported that these additional sequences were related to $MuLV_R$ 70S RNA (15). However, there was still no direct evidence for $MuLV_R$-related proviral sequences in human DNA that had been obtained using viral nucleic acid probes.

The results reported here do offer direct evidence that DNA nucleotide sequences related to $MuLV_R$ can be detected in the DNA from some human tumor tissues. The sequences were found in some tissues of some patients with leukemia, Hodgkin's disease, and multiple myeloma, and they were absent from other tissues of these patients and from tissues of a person with nonneoplastic disease. We recognize that tissues from a normal donor may not be a perfect control for leukemic cells. However, the failure to find the $MuLV_R$-related sequences in the DNA from these tissues, as well as negative results with several tissues from people with neoplasia, is sufficient to indicate that they are not endogenous but must be acquired after fertilization. Moreover, because these results were obtained with DNA, the differences cannot be attributed to variation in gene expression between normal and neoplastic tissues due to difference in growth rates or state of differentiation. These results agree with the finding by Spiegelman and coworkers of extra sequences in leukemic cell DNA not detectable in DNA from normal tissues (4, 36). Other results have recently shown that proviral DNA sequences indistinguishable from some sequences present in the RNA of baboon endogenous virus can be detected in the DNA of some people with leukemia (16, 18). These are in addition to sequences distantly related to the baboon virus RNA present in the DNA of all humans that may be from endogenous human viral sequences (18, 31). These observations led to the conclusion that some humans are infected by a virus very closely related to the baboon virus (18, 37).

The apparent extra nucleotide sequences in certain neoplastic tissues described in this report are related to $MuLV_R$ sequences, but they are not identical to them, because t_e50 differences of 6° were found between hybrids of $MuLV_R$ cDNA and DNA from these samples compared to hybrids formed with the same cDNA and 70S RNA of $MuLV_R$ or with DNA of cells infected by $MuLV_R$. Since $MuLV_R$ and baboon virus are not detectably related (38), these sequences must not be the same as the baboon virus proviral sequences. It appears then that at least two sets of acquired type-C viral DNA sequences are present in human beings, which were transmitted (in the past and/or present) from other species.

Other results suggest that subviral components related to the simian sarcoma virus/gibbon ape leukemia virus group can sometimes be found in human tissues. This includes evidence in some patients with leukemia and preleukemia of a polymerase related to reverse transcriptase of these viruses (5, 6, 8, 14, 41), a protein antigenically related to the virus p30 structural protein (7, 21–23), and some cytoplasmic nucleic acid sequences (11–14). There are also some reports of isolation of replicating viruses highly related to members of this virus group (25–30)

and, more recently, suggestive evidence for antibodies to this virus group in human sera (19, 39, 40). However, proviral sequences related to the simian sarcoma virus/gibbon ape leukemia virus group have not as yet been reported (14). Because $MuLV_R$ 70S RNA shares some sequence homology to RNA of simian sarcoma virus/gibbon ape leukemia virus, it was possible that the sequences related to $MuLV_R$ found in some human tissues were from a virus of this group. However, some of the samples of tumor cell DNA positive with $MuLV_R$ cDNA did not hybridize to simian sarcoma virus cDNA (our unpublished results). Therefore, it is more likely that the origin of the $MuLV_R$-related sequences reported here remains to be identified.

We thank Drs. S. Spiegelman and R. Sweet for examining our cDNA probes and cell DNA, J. Gruber and P. Levine for the supply of tissues, L. Sekely and L. Dabich for supplying the pathology reports, B. Hampar for the use of his laboratory facilities, J. Martin and L. White for reviewing the paper, and J. B. Moloney and J. Gruber of the virus cancer program for helping with virus supply. We are also indebted to our colleagues Drs. D. Gillespie, M. Reitz, C. Saxinger, and F. Wong-Staal for many helpful discussions.

1. Gallo, R. C., Yang, S. S. & Ting, R. C. (1970) *Nature* **228,** 927–929.
2. Sarngadharan, M. G., Sarin, P. S., Reitz, M. S. & Gallo, R. C. (1972) *Nature New Biol.* **240,** 67–72.
3. Baxt, W., Hahlman, R. & Spiegelman, S. (1972) *Nature New Biol.* **240,** 72–75.
4. Baxt, W. & Spiegelman, S. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 3737–3741.
5. Todaro, G. J. & Gallo, R. C. (1973) *Nature New Biol,* **244,** 206–209.
6. Gallagher, R. E., Todaro, G. J., Smith, R. G., Livingston, D. M. & Gallo, R. C. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1309–1313.
7. Sherr, C. J. & Todaro, G. J. (1975) *Science* **197,** 855–857.
8. Mondal, H., Gallagher, R. E. & Gallo, R. C. (1975) *Proc. Natl. Acad. Sci. USA* **71,** 1194–1198.
9. Hehlmann, R., Kufe, D. & Spiegelman, S. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 435–439.
10. Kufe, D., Hehlmann, R. & Spiegelman, S. (1972) *Science* **175,** 182–185.
11. Gallo, R. C., Miller, N. R., Saxinger, W. C. & Gillespie, D. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3219–3224.
12. Miller, N., Saxinger, W. C., Reitz, M. S., Gallagher, R. E., Wu, A. M., Gallo, R. C. & Gillespie, D. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3177–3181.
13. Mak, T. W., Kurtz, S., Manaster, J. & Housman, D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 623–627.
14. Gallo, R. C., Gallagher, R. E., Miller, N. R., Mondal, H., Saxinger, W. C., Mayer, R. J., Smith, R. G. & Gillespie, D. (1975) *Cold Spring Harbor Symp. Quant. Biol.* **39,** 933–961.
15. Baxt, W. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 2853–2857.
16. Reitz, M. G., Miller, N. R., Wong-Staal, F., Gallagher, R. E., Gallo, R. C. & Gillespie, D. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2113–2117.
17. Larsen, C. J., Marty, M., Hamelin, R., Peries, J., Boiron, M. & Tavitian, A. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4900–4904.
18. Wong-Staal, F., Gillespie, D. & Gallo, R. C. (1976) *Nature* **262,** 190–195.
19. Aoki, T., Walling, M. J., Bushar, G. S., Liu, M. & Hsu, K. C. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2491–2495.
20. Sherr, C. J. & Todaro, G. J. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4703–4707.
21. Strand, M. & August, J. T. (1974) *J. Virol.* **13,** 171–180.
22. Mellors, R. C. & Mellors, J. W. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 233–237.
23. Zurcher, C., Brinkhof, J., Bentvelzen, P. & De Man, J. C. H. (1975) *Nature* **254,** 457–459.
24. Stephenson, J. R. & Aaronson, S. A. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1725–1729.
25. Gallagher, R. E. & Gallo, R. C. (1974) *Science* **197,** 350–353.
26. Gallagher, R. E., Salahuddin, S. Z., Hall, W. T., McCredie, K. B. & Gallo, R. C. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4137–4141.
27. Teich, N. M., Weiss, R. A., Salahuddin, S. Z., Gallagher, R. E., Gillespie, D. & Gallo, R. C. (1975) *Nature* **256,** 551–555.
28. Nooter, K., Aarssen, A. M., Bentvelzen, P., d'Groot, F. G. & van Pelt, F. G. (1975) *Nature* **256,** 595–597.
29. Gabelman, N., Waxman, S., Smith, W. & Douglas, S. D. (1975) *Int. J. Cancer* **16,** 355–365.
30. Panem, S., Prochownik, E. V., Reale, F. R. & Kirsten, W. H. (1975) *Science* **189,** 297–298.
31. Benveniste, R. E. & Todaro, G. J. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4513–4518.
32. Benveniste, R. E., Lieber, M. M., Livingston, D. M., Sherr, C. J. & Todaro, G. J. (1974) *Nature* **248,** 17–20.
33. Britten, R. J., Pavich, M. & Smith, J. (1968) *Annual Report Carnegie Institution,* 400–403.
34. Britten, R. J., Graham, D. E. & Neufeld, D. (1974) in *Methods in Enzymology,* eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 29, pp. 363–419.
35. Britten, R. J. & Kohne, D. E. (1968) *Science* **161,** 520–540.
36. Baxt, W., Yates, J. W., Wallace, H. J., Jr., Holland, J. F. & Spiegelman, S. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2629–2632.
37. Gallo, R. C., Wong-Staal, F., Reitz, M., Gallagher, R. E., Miller, N. & Gillespie, D. H. (1976) in *Animal Virology,* eds. Baltimore D., Huang, A. S. & Fox, C. F. (Academic Press, New York), Vol. 4, pp. 385–407.
38. Benveniste, R. E. & Todaro, G. J. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3316–3320.
39. Kurth, R., Teich, N. M., Weiss, R. & Oliver, R. T. D. (1977) *Proc. Natl. Acad. Sci. USA* **74,** in press.
40. Snyder, H. W., Jr., Pincus, T. & Fleissner, E. (1976) *Virology,* **75,** 60–73.
41. Steel, L. K., Laube, H. & Chandra, P. (1976) *Cancer Lett. (Amsterdam),* in press.