Type-C RNA Virus Gene Expression in Human Tissue

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Partially purified fractions of human tissues have been analyzed by competition radioimmunoassay for the presence of two of the principle structural components of type-C RNA viruses, the major core protein (p27 to p30) and the major envelope glycopeptides (gp69/71). Screening of tissues was carried out by use of a heterologous assay system of ^{125}I -labeled Rauscher murine virus p30 antigen and anti-RD 114 virus serum which was found to detect a class of interspecies determinants common to murine, feline, and primate viruses. A competitor with the same apparent affinity for antibody binding as that of purified viral core proteins was found in relatively high concentration in tissues from patients with systemic lupus erythematosus, in some neoplastic tissues, and also in normal human tissues. This competitor from a lupus spleen chromatographed on phosphocellulose and showed size fractionation during gel filtration similar to known p27 to p30 viral proteins. An immunologically reactive protein was also demonstrated by immunodiffusion and by immunoprecipitation of ^{125}I -labeled human protein with anti-RD 114 p28 serum. Analysis of these human competitor proteins with homologous assay systems of viral core proteins and corresponding antisera showed that all, including the normal tissue extracts, appear similar to core proteins of known viruses, especially the RD 114 and woolly monkey species. A hypothesis suggested by these data is that many, if not all, humans harbor at least part of the genome of one or more type-C viruses, the properties of which are similar to those of viruses from other mammalian species, particularly primates.

Evidence for a type-C virus in human cells has been sought in many laboratories by a variety of techniques (for a review, see reference 34). An infectious particle, generally recognized as being of human origin, has not been isolated. Moreover, neither viral nucleic acids nor viral proteins have been detected in normal human cells (5, 6, 7, 14, 20, 40, 46, 48a, 49). In contrast, however, are reports that human myeloblastic leukemia cells contain a viral reverse transcriptase antigenically similar to the woolly monkey virus enzyme (12, 59) and that a variety of human leukemia, sarcoma, and lymphoma tumor cells contain RNA with a sequence homology to murine type-C virus (5, 6). These data would suggest that type-C viruses are a tumorigenic infectious agent (6, 46). Such a proposed role for type-C viruses in humans differs markedly from what might be expected on the basis of results from other animals. Considerable evidence suggests that the type-C virus genome is a normal cell constituent in a variety of animals (23, 55): type-C viruses can be produced in cultured cells from apparently normal

animals (1, 33); DNA homologous to RNA sequences of type-C viruses has been detected in the somatic cells of normal animal tissues (M. M. Lieber and G. J. Todaro, *In F. Becker [ed.], Cancer: A Comprehensive Treatise*, in press); and type-C virus gene proteins have been detected in cells of normal tissues of some animals (M. Strand, F. Lilly, and J. T. August, Cold Spring Harbor Symp. Quant. Biol., in press; 23, 40, 49, 52a).

In view of the implications of this apparent discrepancy between the observed relationship of type-C viruses to human and animal cells, it has become imperative to examine normal as well as malignant human tissues for gene expression of type-C viruses by the most sensitive assays presently available.

A chief problem in assaying for the putative human type-C virus is that its properties cannot be predicted without qualifications. Earlier knowledge of the distinct properties of type-C viruses of different species has led to the expectation that a human type-C virus would be unique. Recently, however, it has been found

that viruses from animals widely separated in evolution possess similar biochemical properties; the endogenous feline (RD 114/CCC group) (11, 35, 36, 45, 57) and baboon viruses (8, 60) are closely related (G. J. Todaro, R. E. Benveniste, R. Callahan, M. M. Lieber, and C. J. Sherr, Cold Spring Harbor Symp. Quant. Biol., in press; R. E. Benveniste and G. J. Todaro, *Nature* [London], in press; 9, 21, 48a, 49), as are the gibbon ape (25) and the woolly monkey viruses (56) as well (Benveniste and Todaro, in press; R. V. Gilden, S. Oroszlan, and M. Hatanka, *Int. Conf. Comp. Virol.*, in press; 9, 42, 48, 61). Another common hypothesis has been that the concentration of type-C viruses (and thus of viral protein) would be significantly greater in malignant tissues as compared to normal tissue. However, of our current experience with mice, this has not proven true. Studies of leukemic AKR and of Lake Casitas wild mice with spontaneous lymphoma showed that the spleen concentrations of viral core or envelope proteins were no more than twofold increased as compared to normal counterparts (Strand et al., Cold Spring Harbor Symp. Quant. Biol., in press; unpublished observations).

We have approached these problems by examining both normal and diseased tissues for the presence of viral proteins. At this time the most sensitive assay procedure in the competition radioimmunoassay of interspecies antigenic determinants common to viral proteins of different species. Wide interspecies antigenic relationships among type-C virus structural proteins have been demonstrated where little or no interspecies nucleic acid sequence homology has been detected (H. M. Temin, *Annu. Rev. Genet.*, in press). The two viral proteins that are most prominent in expressing such interspecies determinants are the major core protein of approximately 30,000 daltons (p27 to p30 [nomenclature as defined in reference 3]) (16) and the chief envelope glycopeptides (gp69/71) (51, 52). Applying these radioimmunoassay procedures and techniques for viral protein purification, we have analyzed several types of human tissues for the presence of p30 and gp69/71 proteins. These studies have included tissues from patients with systemic lupus erythematosus (SLE), as we previously found, in collaboration with Yoshiki and Mellors (64), that tissues and sera of New Zealand black mice, a strain that spontaneously develops connective tissue and autoimmune diseases (22, 28, 37), contain high concentrations of viral proteins.

MATERIALS AND METHODS

Viruses. Rauscher murine type-C virus propagated in the JLS-V9 BALB/c mouse bone marrow culture (63), the Rickard strain of feline type-C virus propagated in the F-422 suspended cell culture derived from the thymus gland of a leukemic cat (44), the endogenous cat type-C virus RD 114 propagated in a human rhabdomyosarcoma cell line (36), and woolly monkey type-C virus propagated in a human lymphoblastoid cell line NC-37 were all provided by S. Mayyasi, D. Larson, and P. Traul (John L. Smith Memorial for Cancer Research). These viruses were propagated, harvested, and purified as previously described (52).

Purification of virus proteins. The Rauscher murine virus gp69/71 and p30, Rickard feline virus p27, RD 114 virus p28, and woolly monkey virus p28 proteins were each purified by phosphocellulose column chromatography and Sephadex gel filtration as described elsewhere (51).

Antisera. Anti-Rauscher virus p30 serum, anti-Rickard virus p27 serum, anti-RD 114 virus p28 serum, and anti-woolly monkey virus p28 serum were all obtained from goats. The animals were immunized with approximately 50 μ g of purified protein in 10 mM BES [*N,N*-bis(2-hydroxyethyl)-2-aminoethane sulfonic acid], pH 6.5, and 1.0 M NaCl mixed with an equal volume of Freund complete adjuvant and injected intramuscularly. Booster injections of approximately 30 μ g of virus proteins were given intramuscularly in Freund incomplete adjuvant at monthly intervals. Anti-RD 114 virus serum (3S-73) and anti-Rickard feline virus serum (1S-121) obtained from goats immunized with purified virus particles disrupted by treatment with Tween ether and pig anti-goat immunoglobulin G serum were generously provided by R. Wilsnack (Huntingdon Research Center, Baltimore, Md.).

Tissues. All tissues were obtained as frozen specimens. A placenta (HF 771) from a 23-year-old woman with SLE and idiopathic thrombocytopenic purpura (ITP) who had been treated with interferon and prednisone, spleen tissue from the same patient following splenectomy, and placenta (HF 772) from another 23-year-old woman with SLE were all generously provided by R. M. McAllister (Children's Hospital of Southern California School of Medicine). A fresh specimen of kidney (A-74-83) from a 7-year-old child with SLE and a hepatocarcinoma were kindly provided by R. M. McAllister, (Children's Hospital of Los Angeles). A SLE kidney (AECOM) stored frozen at -70°C for 3 years was provided by D. Marcus of this institution. A renal adenocarcinoma (hypernephroma) (63-W, 2/22/71) and two transitional cell tumors of the renal pelvis (292WT, 10/10/72 and 95M, 1/3/72) were stored specimens provided by A. Elliott and E. Fraley (University of Minnesota Medical School). A rhabdomyosarcoma (R 519) from a leg of a 63-year-old male, a fibrosarcoma (R 571) from the trunk of a 48-year-old female, a reticulum cell sarcoma (FT 72000241) from a 49-year-old female, the liver (FT 7300356) of a 54-year-old male with lymphosarcoma, a normal kidney (T-044) autopsy speci-

men from a 6-year-old girl who died by drowning, a normal liver autopsy specimen (T-25) from a female with subglottic stenosis, a normal liver (T-28) autopsy specimen from a female with cerebral palsy and pneumonia, and a liver (T-24) from a 9-year-old girl with thrombocytopenic purpura were all obtained through the Office of Program Resources and Logistics, National Cancer Institute. A normal spleen and normal kidney autopsy specimens were obtained at this institution. A dissected normal human trophoblast (RC 74-128) was kindly provided by R. C. Mellors (Hospital of Special Surgery, New York).

Radioimmunoassay. Purified proteins were labeled with ^{125}I as described by Hunter (24).

The reaction mixture for immunoprecipitation contained the following: 0.005 ml of normal goat serum, 0.01 ml containing 1 to 3 ng of ^{125}I -labeled virus protein (10^4 to 10^5 counts/min per ng), and 0.01 ml of diluted goat antiserum as indicated. The final volume was adjusted to 0.2 ml with TEN buffer (20 mM Tris-hydrochloride, pH 7.6, 1 mM EDTA, 100 mM NaCl) containing 2 mg of crystalline bovine serum albumin per ml and 0.2% Triton X-100. All antigen and antibody proteins were diluted in TEN buffer containing 20 mg of crystalline bovine serum albumin per ml. The reaction mixture was incubated at 37°C for 12 to 16 h, after which 0.04 ml of pig anti-goat serum was added to precipitate the antigen-antibody complex. The mixture was then incubated an additional 12 h at 2 to 4°C. Cold TEN buffer (0.5 ml) was added, and the precipitate was collected by centrifugation at 4°C. The pellet was washed twice with 0.5 ml of TEN buffer, and the ^{125}I -labeled antigen present in the precipitate was measured in a gamma counter.

The competition radioimmunoassay reaction mixture was the same except that the dilution of antiserum added was that which precipitated approximately 50% of the labeled antigen, and the competitive inhibition of binding of the ^{125}I -labeled antigen by virus protein was measured. Competing proteins were added immediately before the antiserum. Competing viral purified proteins were diluted in TEN buffer containing 20 mg of crystalline bovine serum albumin per ml and added in 0.01-ml portions. Competing tissue extracts were diluted in TEN buffer containing 2 mg of crystalline serum albumin per ml and 0.2% final concentration of Triton X-100 and added in 0.15-ml portions.

Tissue extraction and phosphocellulose chromatography. Tissue extracts were prepared by mincing the tissues into small fragments and teasing these with forceps into cell suspensions in a minimal volume (8 to 30 mg of protein per ml) of 5 mM Tris-hydrochloride (pH 9.2), 1 mM EDTA, and 400 mM KCl. The cells were suspended by sonic oscillation (Ultrasonic, Heat Systems-Ultrasonic, Inc.), and Triton X-100 was added to a final concentration of 1%. The suspension was then incubated at 37°C for 20 min, frozen three times in dry ice-methanol, and thawed at 37°C. The resulting suspension was centrifuged at $27,000 \times g$ for 10 min. The pellet was extracted twice in the same buffer solution, omitting the KCl. The supernatants were combined and, after

dialysis for 14 h in 3 liters of 10 mM BES (pH 6.5), 1 mM EDTA, and 0.4% Triton X-100, with two changes of dialysate, were applied to a column of phosphocellulose (Whatman P11) previously equilibrated with the same buffer (approximately 10 mg of protein per ml, packed volume). The column was first washed with the dialysis buffer. After the flow through of unadsorbed protein, Triton X-100 was removed by extensive washing of the column with dialysis buffer without Triton X-100 until the absorbance of the effluent was less than 0.02 (λ equals 280 nm). The column was then eluted with buffer solution containing 10 mM BES (pH 6.5), 1 mM EDTA, and KCl, either 1.0 M or as indicated in the text.

RESULTS

Purity of viral protein probes. The ^{125}I -labeled antigens used in the competition radioimmunoassay to measure viral proteins in human cells were the major structural core proteins of type-C viruses, the p30 of Rauscher murine, p27 of Rickard feline, p28 of RD 114, and p28 of woolly monkey viruses, each of which was purified to homogeneity by phosphocellulose column chromatography and Sephadex gel filtration (Fig. 1) (51).

Common antigenic determinants. To choose the most appropriate antigen-antibody combination to be used to screen human tissues by radioimmunoassay, we have characterized the extent of relatedness of the interspecies antigens (15, 16, 41, 47, 52) of purified core proteins of murine, feline, RD 114, and woolly monkey type-C viruses. Analysis by competition radioimmunoassay revealed the presence of several classes of interspecies determinants, some shared by only two species of viruses, others by three, and some by all four proteins (M. Strand and J. T. August, submitted for publication). For purposes of this study, one of the most useful classes appeared to be that detected with Rauscher murine virus p30 as antigen and anti-RD 114 p28 serum. Interspecies determinants common to Rauscher p30 and RD 114 are shared as well with Rickard feline virus and woolly monkey virus proteins at concentrations within 10-fold of each other (Fig. 2). Based on these results, the initial screening of human tissues was carried out with an assay system employing ^{125}I -labeled Rauscher p30 and anti-RD 114 serum.

Partial purification of competing substance from lupus spleen. The most active extract of a number of tissues tested was a spleen from a patient with SLE and ITP. A reduction of 50% in the precipitation of 1 ng of labeled antigen occurred with the addition of

FIG. 1. Polyacrylamide gel electrophoresis of type-C viruses and the purified major structural proteins. Electrophoresis in a linear 5 to 20% polyacrylamide gel gradient in the presence of 0.1% sodium dodecyl sulfate was carried out as described by Baum et al. (4). (1) Standard proteins: cytochrome c (3.3 μ g; mol wt 11,700), chymotrypsinogen (3.3 μ g; mol wt 25,500), ovalbumin (4.2 μ g; mol wt 43,000), bovine serum albumin (2.5 μ g; mol wt 68,000), phosphorylase A (6.6 μ g; mol wt 94,000); (2) Rauscher murine virus (72 μ g of protein); (3) purified Rauscher virus p30 (4 μ g of protein); (4) Theilen strain of feline virus (60 μ g of protein); (5) purified Rickard strain of feline virus p27 (4 μ g of protein); (6) RD 114 virus (75 μ g of protein); (7) purified RD 114 p28 (4.3 μ g of protein); (8) woolly monkey virus (80 μ g of protein); (9) purified woolly monkey virus p28 (4.3 μ g of protein).

approximately 200 μ g of crude extract of this tissue, and at higher concentrations virtually none of the 125 I-labeled murine p30 was precipitated by the antibody (Fig. 3). Moreover, the apparent affinity of the competing material for antibody as judged by the slope of the displacement curve was similar to that of the standard murine p30.

Approximately 50-fold purification of the competing material was achieved by phosphocellulose chromatography, following procedures that had been developed for the purification of the virus p30 protein (51). Most of the

protein in the extract was not adsorbed, whereas the competing material from the SLE-ITP spleen bound and was eluted by an increased concentration of salt (Fig. 3). As judged from the apparent concentration of the competing material in the different fractions, it comprised about 10^{-6} of the total protein of the crude extract. Comparable values for p30 in spleens of normal mice were 10^{-4} for high leukemia strains and 10^{-5} for low leukemia strains (52a).

Additional purification of this competing substance was obtained by Sephadex gel filtra-

FIG. 2. Radioimmunoassay of shared interspecies antigenic determinants of mammalian type-C virus purified major structural proteins (p27 to p30). The competition radioimmunoassay was performed as described with 1 ng of ^{125}I -labeled Rauscher virus p30 (7.4×10^4 counts/min per ng) and a 1:200 final dilution of goat anti-RD 114 p28 serum. Competing purified type-C virus proteins were added as indicated: O, RD 114 p28; $\blacktriangle$, Rickard feline virus p27; Δ , woolly monkey virus p28; $\bullet$, Rauscher murine virus p30. Abbreviations: FeLV, feline leukemia virus; MuLV, murine leukemia virus; WMV, woolly monkey virus.

tion (Fig. 4). The bulk of the protein was present in the void volume of the column. Elution of the competitor was comparable to that of the ^{125}I -labeled murine p30 added as internal standard. As it was necessary to analyze pooled fractions for competitor activity, a precise activity curve was not obtained. However, the molecular weight was within the range of known viral core proteins (27,000 to 30,000), and probably smaller than the murine p30.

Immunoprecipitation with anti-RD 114 p28. Additional evidence for viral proteins in the extract of the SLE-ITP spleen was obtained by labeling the partially purified Sephadex fraction with ^{125}I , incubating the labeled material with monospecific anti-RD 114 p28 serum, and analyzing the immunoprecipitate by polyacrylamide gel electrophoresis. The ^{125}I -labeled protein was first treated with normal goat serum (a prebleeding from the immunized animal). One percent of the radioactivity added (10^5 counts/min) precipitated, and the supernatant was retained. Of this nonspecific precipitate, the majority of the radioactivity (82,000 counts/min) appeared to be in lipids and was removed by acid precipitation and acetone extraction. The remaining material migrated electrophoretically as small-molecular-weight components, with properties of glycolipids (W. L. McLellan, unpublished observations) (Fig. 5A). A portion of the supernatant fraction (4×10^5 acid precipitable counts/min) was treated

with anti-RD 114 p28 serum. About 2×10^4 counts/min was in the immunoprecipitate and 1.2×10^4 counts/min was recovered after acid precipitation and acetone extraction. About 70% of the material migrated electrophoretically, as did the control precipitate, as small-molecular-weight glycolipids. There was, in addition, a labeled component of 30,000 daltons, corresponding in size to a viral core protein (Fig. 5B) and representing 0.01 to 0.1% of the total protein of the Sephadex fraction, in keeping with the value obtained by radioimmunoassay analysis of the fractions. The broadness of the peak may be attributed to more than one protein of different sizes or to diffuse migration in the presence of the large amount of protein in the immune precipitate.

Analysis of different tissues. Several other tissues were tested by the ^{125}I -labeled murine p30-anti-RD 114 serum competition radioim-

FIG. 3. Purification of the competitor of human SLE and ITP spleen. An extract of human SLE and ITP spleen (4.8 gm) was prepared and applied to a phosphocellulose column as described. Most of the protein (79%) was unabsorbed and was recovered in the initial flow through and column wash fractions. The column was eluted with a buffer solution containing 10 mM BES (pH 6.5), 1 mM EDTA, and 500 mM KCl until the adsorbance of the effluent was less than 0.1 (λ equals 280 nm), and then with a solution containing 10 mM BES (pH 6.5), 1 mM EDTA, and 1.0 M KCl. The column fractions were monitored for conductivity and adsorbance at 280 nm, and the fractions were pooled as follows: 0 to 0.3 M KCl, 0.3 to 0.6 M KCl, and 0.6 to 1.0 M KCl. Each of the pooled fractions was precipitated with ammonium sulfate (0 to 75% saturation) and dialyzed against 3 liters of TEN buffer with two changes of dialysate. The 0 to 0.3 M KCl fraction contained 40% of the total phosphocellulose bound protein (473 mg); the 0.3 M to 0.6 M KCl fraction, 49% (476 mg); and the 0.6 M to 1.0 M KCl fraction, 1.6% (16 mg). Activity was measured by radioimmunoassay as described with 1 ng of ^{125}I -labeled Rauscher virus p30 (5.9×10^4 counts/min per ng) and goat anti-RD 114 virus serum (1:7,000 final dilution). Competing purified Rauscher virus p30 or the phosphocellulose fractions were added as indicated (shown as total protein added). Abbreviations: MuLV, murine leukemia virus.

FIG. 4. Determination of the size of the competitor from human SLE and ITP spleen by Sephadex gel filtration. The human SLE and ITP spleen phosphocellulose 1.0 M eluate fraction was prepared as described and concentrated by precipitation with ammonium sulfate (0 to 75% saturation). The precipitate, 4.5 mg of protein, was suspended in 0.2 ml of 10 mM BES (pH 6.5) and 1.0 M NaCl, and 0.1 ng of ^{125}I -labeled Rauscher virus p30 (5×10^4 counts/min per ng) was added. The suspension was then adjusted to final concentration of 5% glycerol, applied to a column (1 by 100 cm) of Sephadex G-75 previously equilibrated with 10 mM BES (pH 6.5) and 1.0 M NaCl, and eluted with the same buffer solution, collecting 1-ml fractions. Each fraction was analyzed for ^{125}I (●), and the absorbance was monitored at 280 nm (O). Fractions corresponding to the major peak of excluded proteins (26 to 34), the peak of ^{125}I -labeled Rauscher virus p30 (35 to 47), and the following fractions (48 to 60, 61 to 75, and 76 to 90) were pooled and concentrated by adding ammonium sulfate to 75% concentration. The precipitates were suspended in 1.0 ml of TEN buffer and dialyzed against 2 liters of TEN buffer with three changes of dialysate. These Sephadex G-75 fractions were analyzed for viral protein by competition radioimmunoassay as described with 3 ng of ^{125}I -labeled woolly monkey virus p28 (1.4×10^4 counts/min per ng) and goat anti-woolly monkey virus p28 serum (1:8,000 final dilution). Activity was found in two fractions as shown and is expressed as total competing protein of the fraction. Abbreviations: MuLV, murine leukemia virus.

munoassay (Table 1). All were processed by phosphocellulose chromatography so as to concentrate and purify a possible type-C viral major structure protein. Strong activity was

FIG. 5. Immunoprecipitate of human SLE and ITP extract by normal and immune sera analyzed by polyacrylamide gel electrophoresis. A portion (0.1 ml) of the human SLE and ITP spleen Sephadex G-75 fraction 35 to 47 (containing 0.001 ng of Rauscher virus p30, 30 counts/min total) was labeled with ^{125}I as described. (A) 100 μl of the ^{125}I -labeled extract (400 ng, 2.5×10^4 counts/min per ng) was added to 50 μl of normal goat serum (preimmunized goat 365) and incubated for 24 h at 0 C; 50 μl of pig anti-goat immunoglobulin G serum was added, and the suspension was incubated overnight at 0 C and then centrifuged at $4,000 \times g$ for 15 min. The supernatant was saved. The precipitate (1×10^5 counts/min) was washed twice with TEN buffer, and the protein was precipitated with 10% trichloroacetic acid, washed with acetone, and applied to a polyacrylamide gel. (B) 50 μl of goat anti-RD 114 p28 serum (goat 365) was added to 100 μl of supernatant from A and incubated for 24 h at 0 C; 40 μl of pig anti-goat immunoglobulin G serum was then added, and the suspension was incubated overnight at 0 C and centrifuged at $4,000 \times g$ for 15 min. The supernatant contained 4×10^5 counts/min. The precipitate (2×10^4 counts/min) was washed twice with TEN buffer, and the protein was precipitated with 10% trichloroacetic acid, washed with acetone, and applied to the polyacrylamide gel. Polyacrylamide gel electrophoresis was carried out as described in the legend to Fig. 1. After electrophoresis, the gel was fixed in 7% acetic acid containing 5% methanol. The individual lanes were cut and then sliced into 2-ml slices with a slab gel cutter (Gordon Instruments). ^{125}I of the fractions was counted in a gamma counter. Viral protein markers shown in A (p15, p30, and gp69/71) were the purified proteins of Rauscher murine virus (53) labeled with ^{125}I as described and added to an additional lane of the same slab gel.

TABLE 1. *Competition of phosphocellulose fractions of human tissues for type-C virus p30 interspecies antigenic determinants*

Tissue ^a	Competitor ^b	
	Competition (%)	Protein (mg)
Placenta, SLE and ITP (HF 771)	50	0.3
Placenta, SLE (HF 772)	45	1.5
Kidney, SLE (A-74-83)	50	0.04
Kidney, SLE (AECOM)	0	1.7
Spleen, SLE and ITP (HF 771)	50	0.017
Liver, ITP (T-24)	5	0.8
Rhabdomyosarcoma (R-519-A)	0	0.4
Renal adenocarcinoma (hypernephroma) (63 ₂ -W)	25	0.45
Renal transitional cell tumor (95 M)	0	1.0
Renal transitional cell tumor (292-WT)	0	0.8
Fibrosarcoma (R 571)	0	3.5
Reticulum cell sarcoma (72-241)	0	2.0
Liver lymphosarcoma (73-356)	15	1.5
Hepatocarcinoma	50	2.0
Kidney, normal (T-044)	30	0.45
Kidney, normal (8/1/73, AECOM)	20	0.4
Spleen, normal (9/10/73, AECOM)	20	1.0
Liver, normal (T-25)	5	0.35
Liver, normal (T-28)	10	0.6
Trophoblast, normal (RC 74-128)	20	1.5

^a Tissue extracts were prepared and fractionated by phosphocellulose ion-exchange chromatography as described. All were a 0 to 1.0 M KCl eluate fraction, except for the SLE placenta (HF 772), which was a 0 to 0.3 M eluate, and the SLE-ITP spleen, which was a 0.6 to 1.0 M eluate.

^b The competition radioimmunoassay was performed as described with 1 ng of ¹²⁵I-labeled Rauscher virus p30 (4×10^4 to 5×10^4 counts/min per ng), goat anti-RD 114 virus serum, and competing tissue extracts as indicated. Competition was quantitated by comparison with the standard competition curve with known amounts of purified Rauscher virus p30 and is expressed as the amount (nanogram per milligram of total protein of the tissue phosphocellulose fraction) required to give 50% reduction in labeled protein precipitation. The values given are either the amount of protein required for 50% competition or the maximal amount of protein tested when the extent of competition was less than 50%.

found in the spleen and placenta of the patient with SLE and ITP, and in kidney tissue of a 7-year-old child with SLE. Another SLE placenta showed less activity but also was positive. These positive tissues were all fresh specimens.

An autopsy specimen of a SLE kidney stored for several years was negative. Positive results, but relatively low levels of activity, were observed with a renal adenocarcinoma, a lymphosarcoma, and a hepatocarcinoma. No competition was detected with fractions from several other neoplasia.

All of the normal tissues, spleen, liver, kidney, and placenta also showed competition comparable to that of the positive neoplastic tissues.

It should be emphasized that these results were obtained with partially purified fractions, 10- to 50-fold enriched, assuming complete recovery of competitor. In several cases, competition was also tested with crude extracts and was not observed, even when a 10-fold greater amount of protein was added.

Competition in homologous assay systems.

The relationship of the competing material to known type-C viruses was analyzed by use of homologous assay systems of core proteins purified from murine, feline, RD 114 and woolly monkey viruses and their respective monospecific antisera. In such systems, the antibodies bind type- and group-specific as well as the interspecies antigenic determinants of the proteins. Competition of greater than 50% with an affinity equal to that of the standard antigen has been shown to be indicative of proteins highly similar to the antigen (52).

Theoretically, if a viral protein of a new and distinctive human virus were detected by the heterologous interspecies assay systems (¹²⁵I-labeled murine p30-anti-RD 114 serum), this protein would not bind the group-specific and type-specific antibodies of antiserum homologous to the analogous protein of a different virus. The only fraction that possibly demonstrated such a pattern was that from the placenta of the patient with lupus erythematosus and thrombocytopenic purpura; greater competitor activity was detected in the heterologous system than with any of the homologous systems (Table 2). With all other tissues, the competing activity appeared to correspond to proteins of other known mammalian type-C viruses.

The general pattern for all of the tissues, including the normal organs, was for strong activity for both the RD 114 and woolly monkey virus groups. There were also low levels of competing activity for antibodies of the murine group; in this case, the apparent affinity of antibody binding was reduced as compared to the standard Rauscher p30, a pattern similar to that seen with other types of murine viruses in this assay system (52). An exception to the

TABLE 2. Analysis of human tissue fractions for group-specific antigenic determinants of type-C proteins

Tissue phosphocellulose fractions ^a	Competitor (ng/mg of total protein) measured by homologous assay systems ^b			
	MuLV p30 × anti-MuLV p30	FeLV p27 × anti-FeLV p27	RD 114 p28 × anti-RD 114 p28	WMV p28 × anti-WMV p28
Placenta, SLE and ITP (HF 771)	<1 (35%, 0.9 mg)	<1 (0%, 2.5 mg)	<1 (40%, 2.5 mg)	<1 (20%, 2.5 mg)
Kidney, SLE (A-74-83)	28, 17, 10 ^c	NT	<1 (5%, 1 mg)	NT
Spleen, SLE and ITP (HF 771)	<1 (35%, 0.9 mg)	200	1200	900
Liver, ITP	<1 (35%, 2.5 mg)	NT	4	12
Hepatocarcinoma	<1 (25%, 2.5 mg)	<1 (0%, 6.9 mg)	15	3
Kidney, normal (T-044)	2	NT	83	75
Kidney, normal (8/1/73, AECOM)	2	NT	7	8
Spleen, normal (9/10/73, AECOM)	<1 (25%, 0.8 mg)	NT	17	17
Liver, normal (T-25)	<1 (25%, 1.0 mg)	NT	3	15
Liver, normal (T-28)	1	NT	7	8
Trophoblast, normal (RC 74-128)	1	NT	<1 (0%, 4.5 mg)	<1 (35%, 1.5 mg)

^a Tissue extracts were prepared and fractionated by phosphocellulose ion-exchange chromatography as described.

^b The homologous competition radioimmunoassay was performed as described with the following reagents as indicated: 2 ng of ¹²⁵I-labeled Rauscher virus p30 (6×10^4 counts/min per ng) and goat anti-Rauscher virus p30 (1:60,000 final dilution), or 3 ng of ¹²⁵I-labeled Rickard feline virus p27 (1.5×10^4 counts/min per ng) and goat anti-Rickard feline virus p27 serum (1:1,200 final dilution), or 3 ng of ¹²⁵I-labeled RD 114 p28 (3×10^4 counts/min per ng) and goat anti-RD 114 p28 serum (1:6,000 final dilution), or 3 ng of ¹²⁵I-labeled woolly monkey virus p28 (1.4×10^4 counts/min per ng) and goat anti-woolly monkey virus serum (1:10,000 final dilution). Competition was quantitated by comparison with the standard competition curve with known amounts of purified unlabeled viral protein corresponding to the ¹²⁵I-labeled protein and expressed as the amount (nanograms per milligram of total protein of the tissue phosphocellulose fraction) required to give 50% reduction in labeled protein precipitation. Numbers in parentheses after competitor concentrations of <1% indicate the percentage of reduction in labeled protein precipitation obtaining with the maximal amount of phosphocellulose fraction tested. Abbreviations: FeLV, feline leukemia virus; MuLV, murine leukemia virus; WMV, woolly monkey virus; NT, not tested.

^c Multiple determinations with the same tissue fraction.

general pattern was a SLE kidney, which showed the bulk of activity in the murine assay system, and little if any in the RD 114 system.

The phosphocellulose purified SLE-ITP spleen fractions showed exceptionally strong competition in the RD 114 and woolly monkey virus assay; activity in the feline assay, fivefold lower, was also strong. Low activity was also observed in the murine assay system, but in this case the apparent affinity of the competitor for the antibodies was reduced, and the inhibition could be interpreted as binding of interspecies determinants.

The activity of the normal tissue was vividly demonstrated in the primate assay systems. The actual inhibition curves of these assays showed an apparent affinity for antibody binding highly similar to the standard test antigen in both the RD 114 (Fig. 6) and the woolly monkey virus system (data not shown). Moreover, in both, there was complete competition with high concentrations of the more active

fractions, indicating a very close similarity of the human competing proteins and those of these two viruses. Because of the close relatedness of the RD 114 to the baboon virus p30, it can safely be expected that these human tissue extracts could be positive in a baboon virus homologous assay as well. By contrast, in such assays, murine or feline virus proteins show a much lower degree of relatedness, competing only for antibodies directed against interspecies determinants with low affinity (Fig. 6 and unpublished observations).

Immunodiffusion. In an attempt to further demonstrate the immunological properties of the competitors, immunodiffusion analysis was carried out with the SLE and ITP spleen phosphocellulose fraction concentrated ninefold by ammonium sulfate precipitation. Weak, but readily discernable, immunoprecipitates were observed with anti-RD 114 p28 serum and anti-woolly monkey virus p28 serum, suggesting a close relatedness of the human competitors to

Fig. 6. Analysis of the competition of partially purified fractions from normal human tissues in the RD 114 p28 homologous assay system. The competition radioimmunoassay was performed as described with 3 ng of ^{125}I -labeled RD 114 p28 (3.0×10^4 counts/min per ng), goat anti-RD 114 p28 serum (final dilution 1:6,000), and competing purified RD 114 p28, purified Rauscher virus p30, or phosphocellulose fractions of human tissue extracts, shown as total protein added. The normal tissues added were as follows: $\circ$, kidney (T-044); Δ , spleen (AECOM); $\square$, kidney (AECOM); $\blacktriangle$, liver (T-28); $\bullet$, liver (T-25); $\blacksquare$, trophoblast (RC 74-128).

these viruses, in accord with Table 2 (Fig. 7). No immunoprecipitates were detected with high-titer anti-FeLV serum (1S-121) or anti-Rauscher MuLV p30 serum (results not shown). The anti-woolly monkey virus p28 serum gave little precipitation of RD 114 p28, as there is little relatedness between these two viruses. In comparison with the precipitin band demonstrated by 2 μg of RD 114 p28, it is estimated that of the 350 μg of human protein applied, less than 0.5 μg was precipitated.

Expression of viral envelope glycopeptides.

The presence of material competing for the interspecies determinants of the major viral glycopeptides (gp69/71) was also examined. This assay system was composed of ^{125}I -labeled Rauscher gp69/71 and anti-Theilen feline virus serum. In previous studies of the expression of type-C virus proteins in tissues of different strains of mice, we had found that both the p30 and gp69/71 could be detected in virtually all mice and that the expression of these two proteins varied markedly; between different strains the ratios of concentrations of those two proteins differed as much as 100-fold (Strand et al., Cold Spring Harbor Symp. Quant. Biol., in press; 52a).

With all of the tissues tested, including the SLE kidney found negative for p30 competition, there was apparent competing material with a lower antibody affinity than the standard murine or feline virus envelope proteins (data not shown). Further analysis of this viral protein

awaits the purification and development of assay systems for primate virus envelope glycopeptides.

DISCUSSION

Characterization of the partially purified competitor from the spleen of a patient with SLE and ITP showed that the extract contained protein(s) of approximately 30,000 daltons that bound to antibodies directed against RD 114 and woolly monkey virus core proteins with the same affinity as the viral proteins. The results provide strong evidence for the presence of viral protein(s) in human tissue. Because of the small amount of competitor detected in normal tissues, less than 1 ng per mg of protein of the crude extract, a similar chemical analysis of the competitor of normal or malignant tissues was not carried out. In this case identification of the competitor as a viral protein is based upon its apparent equal affinity for antibody binding as the purified viral proteins and upon the specificity of the competition to reactions employing primate virus proteins. These findings support a hypothesis that many, if not all, humans contain the genetic information for proteins analogous to those of one or more type-C viruses of other mammalian species, particularly primates. Ultimate proof of this model should include the complete purification and characterization of these putative viral proteins.

The relatedness of the human proteins to type-C viruses of other animal species, simian and the endogenous cat and baboon viruses, and probably feline and murine viruses as well, is a remarkable finding. The presence of multiple different group-specific determinants in tissues of the same animal has not previously been observed (Benveniste and Todaro, in press; 7, 9, 48a, 49, 50); however, this possibility has not been tested at the same level of tissue protein purity employed in these studies. Apparent alternative explanations for the results are: (i) that humans harbor several type-C viruses similar to those present in other species, or (ii) that type-C viruses of humans are distinct but contain one or more proteins with immunological properties similar to those of other viruses, perhaps arising from earlier genetic recombination as has been suggested for antigenic variation of influenza virus (29, 30). The findings are not attributable to the properties of the viral proteins used as probes in the radioimmunoassay; extensive studies of these purified proteins and the antisera used in this study have shown that competition for binding of a protein and its homologous antiserum by any of the other pro-

FIG. 7. Gel diffusion analysis of human SLE and ITP spleen fractions and RD 114 p28 protein. Immunodiffusion plates were prepared with microscope slides (75 by 35 mm) coated with 4.5 ml of 0.8% agarose in 50 mM Tris (pH 7.2), 100 mM NaCl, and 0.5 M glycine, and wells (25 μ liter volume) were cut immediately prior to use. The antisera added were (A) goat anti-RD 114 p28 and (C) goat anti-woolly monkey virus p28. The antigens were (B) 2.0 μ g of RD 114 p28 and (D) 350 μ g of human SLE and ITP spleen phosphocellulose 1.0 M eluate fraction as described. The fraction had been concentrated ninefold by precipitation with ammonium sulfate (0 to 75% saturation), suspended in TEN buffer, and dialyzed against TEN buffer. The gel was dried and then stained with 0.1% Buffalo Black.

teins is weak and limited to an inhibition of labeled antigen precipitation of approximately 30% or less, reflecting a fraction of cross-reac-

tive interspecies antigens (Strand and August, unpublished observations).

The presence of a unique human type-C virus

would have been demonstrated in this study by an inhibition of antibody precipitation with the heterologous assay system but not with any of the homologous systems. Convincing evidence for such a virus was not found, as such a pattern was observed only with one tissue (SLE and ITP placenta [HF 771]) of the many tested. There are several possible explanations for the absence of such an apparent unique human virus protein in the other tissues. Such a virus or viral proteins may be present but at a concentration below that presently detectable. It is also possible that there is a unique human virus in high concentrations, but that the core proteins of such a human virus do not show the interspecies determinants common to the murine, feline, RD 114 and woolly monkey virus proteins and would thus not have been detected. Another alternative is that there is no distinct human type-C virus markedly different from primate viruses. Those considerations have considerable bearing on the search for infectious type-C virus from human tissues. It can no longer be assumed that such a virus can easily be distinguished from other animal viruses, as, for example, in the controversy over the ESP virus (13, 17, 43, 48). It is also possible that human cells may yield recombinant or pseudotype viruses with proteins derived from different species of viruses.

Whether these viral proteins reflect the occurrence of infectious virus spread from man to man or from animal to man, or of an endogenous virus, is yet to be determined. As the genome of type-C viruses is found in normal tissues of several animals (10, 11, 27, 31, 32, 33, 39, 54, 58, 62) and is speculated to be transmitted from parent to progeny as other cellular genes (23) or to evolve from cellular processes of information transfer from DNA to RNA to DNA (55), it is reasonable to suspect that the same occurs in humans as well. The finding of viral gene products in tissues of apparently normal humans is consistent with this hypothesis. However, viral nucleic acid in tissues of normal humans has not been detected. The reason for this discrepancy is not clear, but it is possible that the extent of nucleic acid homology implicit in the antigenic cross-reactivity is too little to be detected in nucleic acid hybridization experiments.

Of the limited number of malignant tissues tested, none contained a greater concentration of viral protein than did the normal cells. It therefore appeared that the expression of some type-C virus gene products in humans is not unique to malignant tissue. This is in accord with the expression of virus proteins in normal mouse tissues as well (Strand et al., Cold

Spring Harbor Symp. Quant. Biol., in press; 52a). Moreover, additional studies have shown that the spleen concentration of viral p30 of leukemic AKR mice was increased less than two-fold as compared to normal litter mates (Strand et al., Cold Spring Harbor Quant. Biol., in press). It remains to be determined how these results relate to other reports indicating an increased level of viral nucleic acid (5, 6) or reverse transcriptase activity (14) specifically in human malignant tissues.

A possible role of viruses in the etiology of human SLE has been suggested by electron microscopy studies demonstrating structures resembling viruses in tissues of patients with this disease (R. Fresco, Fed. Proc. 27:246; 18, 19, 26). With the New Zealand black strain of mouse there is a close correlation between the occurrence and severity of a lupus syndrome, the presence of the endogenous type-C virus, and the deposition of viral protein as an immune complex in tissues of the animal (R. C. Lerner, F. J. Dixon, B. P. Croker, B. C. DelVillano, F. C. Jensen, S. J. Kennel, and P. J. McConahey, Advan. Biosci., in press; 38, 64). The present findings of a markedly increased concentration of the virus core protein in tissues of humans with SLE provides additional evidence for the involvement of type-C virus in human autoimmune diseases.

At the present time, animal model systems indicate a possible correlation of type-C viruses with neoplasia (23), autoimmune diseases (Lerner et al., in press; 38) and central nervous system diseases (2). The results reported here suggest that purified viral proteins provide a means to test for possible correlations of viral protein concentration or antibodies to these proteins to analogous human diseases.

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ADDENDUM IN PROOF

In recent studies, C. Sherr and C. Todaro (in press) analyzed partially purified extracts from 33 human tumors for competing antigens in a radioimmunoassay for the baboon p30 protein. A competitor

cross-reactive with the p30 protein of the RD 114 virus but not with several other mammalian type-C viruses was detected in two of the extracts.

LITERATURE CITED

1. Aaronson, S. A., G. J. Todaro, and E. M. Scolnick. 1971. Induction of mouse C-type viruses from clonal lines of virus-free BALB/3T3 cells. *Science* **174**:157-159.
2. Andrews, J. M., and M. B. Gardner. 1974. Lower motor neuron degeneration associated with type-C RNA virus infection in mice: neuropathological features. *J. Neuropathol. Exp. Neurol.* **33**:285-296.
3. August, J. T., D. P. Bolognesi, E. Fleissner, R. V. Gilden, and R. C. Nowinski. 1974. A proposed nomenclature for the virion protein of oncogenic RNA viruses. *Virology* **60**:595-600.
4. Baum, S. G., M. S. Horwitz, and J. V. Maizel, Jr. 1972. Studies of the mechanism of enhancement of human adenovirus infection in monkey cells by simian virus 40. *J. Virol.* **10**:211-219.
5. Baxt, W. G., and S. Spiegelman. 1972. Nuclear DNA sequences present in human leukemic cells and absent in normal leukocytes. *Proc. Nat. Acad. Sci. U.S.A.* **69**:3737-3741.
6. Baxt, W., J. W. Yates, H. J. Wallace, Jr., J. F. Holland, and S. Spiegelman. 1973. Leukemia-specific DNA sequences in leukocytes of the leukemic member of identical twins. *Proc. Nat. Acad. Sci. U.S.A.* **70**:2629-2632.
7. Benveniste, R. E., R. Heinemann, G. L. Wilson, R. Callahan, and G. J. Todaro. 1974. Detection of baboon type C viral sequences in various primate tissues by molecular hybridization. *J. Virol.* **14**:56-57.
8. Benveniste, R. E., M. M. Lieber, D. M. Livingston, C. J. Sherr, G. J. Todaro, and S. S. Kalter. 1974. Infectious type-C virus isolated from a baboon placenta. *Nature (London)* **248**:17-20.
9. Benveniste, R. E., and G. J. Todaro. 1973. Homology between type-C viruses of various species as determined by molecular hybridization. *Proc. Nat. Acad. Sci. U.S.A.* **70**:3316-3320.
10. Breese, S. S., Jr. 1970. Virus-like particles occurring in cultures of stable pig kidney cell lines. *Arch. Gesamte Virusforsch.* **30**:401-404.
11. Fischinger, P. J., P. T. Peebles, S. Nomura, and D. K. Haapala. 1973. Isolation of an RD-114-like oncornavirus from a cat cell line. *J. Virol.* **11**:978-985.
12. Gallagher, R. E., G. J. Todaro, R. G. Smith, D. M. Livingston, and R. C. Gallo. 1974. Relationship between RNA-directed DNA polymerase (reverse transcriptase) from human acute leukemic blood cells and primate type-C viruses. *Proc. Nat. Acad. Sci. U.S.A.* **71**:1309-1313.
13. Gallo, R. C., P. S. Sarin, P. T. Allen, W. A. Newton, E. S. Priori, J. M. Bowen, and L. Dmochowski. 1971. Reverse transcriptase found in type-C virus particles of human origin. *Nature (London)* **232**:140-142.
14. Gallo, R. C., S. S. Yang, and R. C. Ting. 1970. RNA dependent DNA polymerase of human acute leukaemic cells. *Nature (London)* **228**:927-929.
15. Geering, G., W. D. Hardy, Jr., L. J. Old, E. de Harven, and R. S. Brodey. 1968. Shared group-specific antigen of murine and feline leukemia viruses. *Virology* **36**:678-707.
16. Gilden, R. V., S. Oroszlan, and R. J. Huebner. 1971. Coexistence of intraspecies specific antigenic determinants on the major structural polypeptides of mammalian C-type viruses. *Nature N. Biol.* **231**:107-108.
17. Gilden, R. V., N. P. Parks, G. J. Todaro, and R. Huebner. 1971. Group specific antigen in human cell lines containing C-type virus. *Nature (London)* **233**:102-103.
18. Grimley, P. M., J. L. Decker, H. J. Michelitch, and M. M. Frantz. 1973. Abnormal structures in circulating lymphocytes from patients with systemic lupus erythematosus and related diseases. *Arthritis Rheum.* **16**:313.
19. Haas, Y. E., and E. J. Yunis. 1970. Tubular inclusions of systemic lupus erythematosus. Ultrastructural observations regarding their possible viral nature. *Exp. Mol. Pathol.* **12**:257-263.
20. Hehlmann, R., D. Kufe, and S. Spiegelman. 1972. RNA in human leukemic cells related to the RNA of a mouse leukemia virus. *Proc. Nat. Acad. Sci. U.S.A.* **69**:435-439.
21. Hellman, A., P. T. Peebles, J. E. Strickland, A. K. Fowler, S. S. Kalter, S. Oroszlan, and R. V. Gilden. 1974. Baboon virus isolate M-7 with properties similar to feline virus RD 114. *J. Virol.* **14**:133-138.
22. Howie, V. B., and B. J. Helyer. 1968. The immunology and pathology of NZB mice. *Advan. Immunol.* **9**:215-266.
23. Huebner, R. J., and G. J. Todaro. 1969. Oncogenes of RNA tumor viruses as determinants of cancer. *Proc. Nat. Acad. Sci. U.S.A.* **64**:1087-1094.
24. Hunter, W. M. 1967. The preparation of radioiodinated proteins of high activity, their reaction with antibody in vitro: the radioimmunoassay, p. 608-642. In D. M. Weir (ed.), *Handbook of experimental immunology*. F. A. Davis Co., Philadelphia.
25. Kawakami, T. G., S. D. Huff, P. M. Buckley, D. L. Dungworth, S. P. Snyder, and R. V. Gilden. 1972. C-type virus associated with gibbon lymphosarcoma. *Nature N. Biol.* **235**:170-171.
26. Kawano, K., L. Miller, and P. Kimmelstiel. 1969. Virus-like structures in lupus erythematosus. *N. Engl. J. Med.* **281**:1228-1233.
27. Klement, V., M. O. Nicolson, and R. J. Huebner. 1971. Rescue of the genome of focus forming virus from rat non-productive lines by 5'-bromodeoxyuridine. *Nature N. Biol.* **234**:12-14.
28. Lambert, P., and F. Dixon. 1968. Pathogenesis of the glomerulonephritis of NZB/W mice. *J. Exp. Med.* **127**:507-522.
29. Laver, W. G., J. C. Downie, and R. G. Webster. 1974. Studies on antigenic variation in influenza virus. Evidence for multiple antigenic determinants on the hemagglutinin subunits of A/Hong Kong/68 (H3 N2) virus and the A/England/72 strains. *Virology* **59**:230-244.
30. Laver, W. G., and R. G. Webster. 1973. Studies on the origin of pandemic influenza. III. Evidence implicating duck and equine influenza viruses as possible progenitors of the Hong Kong strain of human influenza. *Virology* **51**:383-391.
31. Lieber, M. M., R. E. Benveniste, D. M. Livingston, and G. J. Todaro. 1973. Mammalian cells in culture frequently release type-C viruses. *Science* **182**:56-59.
32. Livingston, D. M., and G. J. Todaro. 1973. Endogenous type-C virus from a cat clone with properties distinct from previously described feline type-C viruses. *Virology* **53**:142-151.
33. Lowy, D. R., W. P. Rowe, N. Teich, and J. W. Hartley. 1971. Murine leukemia virus: high frequency activation in vitro by 5-iododeoxyuridine and 5-bromodeoxyuridine. *Science* **174**:155-156.
34. McAllister, R. M. 1973. Viruses in human carcinogenesis. *Progr. Med. Virol.* **16**:48-85.
35. McAllister, R. M., M. Nicolson, M. B. Gardner, S. Rasheed, R. W. Rongey, W. D. Hardy, Jr., and R. V. Gilden. 1973. RD-114 virus compared with feline and murine type-C viruses released from RD cells. *Nature N. Biol.* **242**:75-78.
36. McAllister, R. M., M. Nicolson, M. B. Gardner, R. W. Rongey, S. Rasheed, P. S. Sarma, R. J. Huebner, M. Hatanaka, S. Oroszlan, R. V. Gilden, A. Kabigting,

- and L. Vernon. 1972. C-type virus released from cultured human Rhabdomyosarcoma cells. *Nature N. Biol.* **235**:3-6.
37. Mellors, R. C. 1966. Autoimmune and immunoproliferative diseases of NZB mice and hybrids. *Int. Rev. Exp. Pathol.* **5**:217.
 38. Mellors, R. C. 1968. Autoimmune disease and neoplasia of NZB mice—implication of murine leukemia-like virus. *Perspect. Virol.* **6**:239-256.
 39. Moennig, V., H. Frank, G. Hunsmann, P. Ohms, H. Schwarz, W. Schäfer, and H. Strandström. 1974. C-type particles produced by a permanent cell line from a leukemia pig. II. Physical, chemical and serological characterization of the particles. *Virology* **57**:179-188.
 40. Parks, W. P., D. M. Livingston, G. J. Todaro, R. E. Benveniste, and E. M. Scolnick. 1973. Radioimmunoassay of mammalian type-C viral proteins. III. Detection of viral antigen in normal murine cells and tissues. *J. Exp. Med.* **137**:622-635.
 41. Parks, W. P., and E. M. Scolnick. 1972. Radioimmunoassay of mammalian type-C viral proteins: interspecies antigenic reactivities of the major internal polypeptide. *Proc. Nat. Acad. Sci. U.S.A.* **69**:1766-1770.
 42. Parks, W. P., E. M. Scolnick, M. C. Noon, C. J. Watson, and T. G. Kawakami. 1973. Radioimmunoassay of mammalian type-C polypeptides. IV. Characterization of Woolly Monkey and gibbon viral antigens. *Int. J. Cancer* **12**:129-137.
 43. Priori, E. S., L. Dmochowski, B. Myers, and J. R. Wilbur. 1971. Constant production of type-C virus particles in a continuous tissue culture derived from pleural effusion cells of a lymphoma patient. *Nature (London)* **232**:61-62.
 44. Rickard, C. G., J. E. Post, F. Noronha, and L. M. Barr. 1969. A transmissible virus induced lymphocytic leukemia of the cat. *J. Nat. Cancer Inst.* **42**:987-1014.
 45. Sarma, P. S., J. Tseng, Y. K. Lee, and R. V. Gilden. 1973. Virus similar to RD-114 virus in cat cells. *Nature N. Biol.* **244**:56-58.
 46. Sarngadharan, M. G., P. S. Sarin, M. S. Reitz, and R. C. Gallo. 1972. Reverse transcriptase activity of human acute leukaemic cells: purification of the enzyme, response to AMV 70S RNA, and characterization of the DNA product. *Nature N. Biol.* **240**:69-72.
 47. Schäfer, W., P. J. Fischinger, J. Lange, and L. Pister. 1972. Properties of mouse leukemia viruses. I. Characterization of various antisera and serological identification of viral components. *Virology* **47**:197-209.
 48. Scolnick, E. M., W. P. Parks, G. J. Todaro, and S. A. Aaronson. 1972. Immunological characterization of primate C-type virus reverse transcriptases. *Nature N. Biol.* **235**:35-40.
 - 48a. Sherr, C. J., R. E. Benveniste, and G. J. Todaro. 1974. Type-C viral expression in primate tissues. *Proc. Nat. Acad. Sci. U.S.A.* **71**:3721-3725.
 49. Sherr, C. J., and C. J. Todaro. 1974. Radioimmunoassay of the major group specific protein of endogenous baboon type-C viruses: relation to the RD 114/CCC group and detection of antigen in normal baboon tissues. *Virology* **61**:168-181.
 50. Stephenson, J. R., and S. A. Aaronson. 1973. Expression of endogenous RNA C-type virus group-specific antigens in mammalian cells. *J. Virol.* **12**:564-569.
 51. Strand, M., and J. T. August. 1973. Structural proteins of oncogenic ribonucleic acid viruses. *Interspec. II, a new interspecies antigen.* *J. Biol. Chem.* **248**:5627-5633.
 52. Strand, M., and J. T. August. 1974. Structural proteins of mammalian oncogenic RNA viruses: multiple antigenic determinants of the major internal protein and envelope glycoprotein. *J. Virol.* **13**:171-180.
 - 52a. Strand, M., F. Lilly, and J. T. August. 1974. Host control of endogenous murine leukemia virus gene expression: concentration of viral proteins in high and low leukemia mouse strains. *Proc. Nat. Acad. Sci. U.S.A.* **71**:3682-3686.
 53. Strand, M., R. Wilsnack, and J. T. August. 1974. Structural proteins of mammalian oncogenic RNA viruses: immunological characterization of the p15 polypeptide of Rauscher murine virus. *J. Virol.* **14**:1575-1583.
 54. Strandström, H., P. Veijalainen, V. Moennig, G. Hunsmann, H. Schwarz, and W. Schäfer. 1974. C-type particles produced by a permanent cell line from a leukemic pig. I. Origin and properties of the host cells and some evidence for the occurrence of C-type-like particles. *Virology* **57**:175-178.
 55. Temin, H. M. 1972. The RNA tumor viruses—background and foreground. *Proc. Nat. Acad. Sci. U.S.A.* **69**:1006-1020.
 56. Theilen, G. H., D. Gould, M. Fowler, and D. L. Dungworth. 1971. C-type virus in tumor tissue of a Woolly Monkey (*Lagothrix* spp.) with fibrosarcoma. *J. Nat. Cancer Inst.* **17**:881-889.
 57. Todaro, G. J., R. E. Benveniste, M. M. Lieber, and D. M. Livingston. 1973. Infectious type-C viruses released by normal cat embryo cells. *Virology* **55**:505-515.
 58. Todaro, G. J., R. E. Benveniste, M. M. Lieber, and C. J. Sherr. 1974. Characterization of a type-C virus released from the porcine cell line PK(15). *Virology* **58**:65-74.
 59. Todaro, G. J., and R. C. Gallo. 1973. Immunological relationship of DNA polymerase from human acute leukaemia cells and primate and mouse leukaemia virus reverse transcriptase. *Nature (London)* **244**:206-209.
 60. Todaro, G. J., C. J. Sherr, R. E. Benveniste, M. M. Lieber, and J. L. Melnick. 1974. Type-C viruses of baboons: isolation from normal cell cultures. *Cell* **2**:55-61.
 61. Tronick, S. R., J. R. Stephenson, and S. A. Aaronson. 1974. Comparative immunological studies of RNA C-type viruses: radioimmunoassay for a low molecular weight polypeptide of Woolly Monkey leukemia virus. *Virology* **57**:347-356.
 62. Weiss, R. A., R. R. Friis, E. Katz, and P. K. Vogt. 1971. Induction of avian tumor viruses in normal cells by physical and chemical carcinogens. *Virology* **46**:920-938.
 63. Wright, B. S., P. A. O'Brien, G. P. Shibley, S. A. Mayyasi, and J. C. Lasfargues. 1967. Infection of an established mouse bone marrow cell line (JLS-V9) with Rauscher and Moloney murine leukemia viruses. *Cancer Res.* **27**:1672-1677.
 64. Yoshiki, T., R. C. Mellors, M. Strand, and J. T. August. 1974. The viral envelope glycoprotein of murine leukemia virus and the pathogenesis of immune-complex glomerulonephritis of New Zealand mice. *J. Exp. Med.* **140**:1011-1027.