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Type C Viral Expression in Primate Tissues

(baboon type C virus/RNA·DNA hybridization/p30 antigen/virogene evolution)

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ABSTRACT **Nucleic acid sequences homologous to a [^{3}H]DNA transcript prepared from an endogenous baboon type C virus are readily detected in both the cellular DNA and RNA of several different Old World monkeys. Competitive radioimmunoassays for the major viral protein (p30) of baboon type C viruses detect viral antigen in a normal stumptail spleen and a rhesus ovarian carcinoma. The p30 antigen from these tissues is closely related by several immunologic criteria to the p30 protein of baboon type C viruses. The results indicate that normal primate tissues transcribe endogenous viral-specific RNA and translate at least one viral structural protein.**

Six type C viruses have been isolated from separate, apparently "virus-negative," baboon tissues and cultured cell lines (1, 2). These baboon type C viruses are related to one another (2, 3), and can be distinguished from previously defined classes of mammalian type C viruses (1, 3, 4). Sequences homologous to the genomes of baboon type C viruses have been found in the DNA of tissues of normal baboons obtained from several different colonies (5). Certain normal baboon tissues have also been shown to be expressing low levels of viral RNA (1, 5) and a major viral structural protein (p30) (3). These results support the conclusion that the baboon type C viruses are endogenous, vertically transmitted viruses of primates.

Sequences related to baboon type C viral RNA have been detected in the DNA of other Old World monkeys (patas, rhesus, stumptail, and African green). Hybrids formed between [^{3}H]DNA transcripts of baboon type C viral RNA and Old World monkey cellular DNA show a greater degree of base pair mismatching than do hybrids obtained with baboon cellular DNA, indicating that the endogenous virogenes in these related species have evolved away from one another (5,6) during the 15 million years since the species diverged (7).

Sequences homologous to two type C viruses isolated from tumors of a woolly monkey (8) and gibbon ape (9) cannot be detected in the DNA of normal primate tissues (10, 5), while related sequences are found in the DNA of normal mice (5). These findings suggest that these viruses originated from endogenous xenotropic viruses of mice (11–14), or a close rodent relative of the mouse, and are, thus, not endogenous viruses of primates.

The studies reported here indicate that tissues from Old World monkeys extensively transcribe viral specific RNA. Some of these tissues also produce viral p30 proteins that are closely related immunologically to the homologous protein of the baboon type C viruses. Primates other than the baboon, then, have virogene sequences in their DNA and express type C virus-specific information in at least some of their tissues.

MATERIALS AND METHODS

Cells and Culture Conditions. Cell lines used were: a canine thymus line, FCf2Th, from the Naval Biomedical Research Laboratory (Oakland, Calif.); the human rhabdomyosarcoma, A204 (15); a line of human embryonic fibroblasts, M413; the normal rat kidney line, NRK (16); a continuous line of gibbon lymphosarcoma cells (9); and the murine cell line, NIH/3T3 (17). All lines were grown and subcultured as described (2).

Viruses. The baboon viruses, M7 and BAB8-Lg (1, 2), were grown in either the FCf2Th or the A204 cell lines. RD-114 virus was grown in RD cells (18) at Pfizer Laboratories (Maywood, N.J.). Rauscher murine leukemia virus (R-MuLV) was from ElectroNucleonics Laboratories (Silver Spring, Md.). S2CL3 is an endogenous mouse type C virus produced spontaneously by a morphologically transformed BALB/3T3 cell line (19). The Gardner–Arnstein strain of feline leukemia-sarcoma virus (FeLV–FSV) was obtained from virus-producing cat cell cultures (Naval Biomedical Research Laboratories) and was grown in canine FCf2Th cells. The woolly monkey type C virus (SSAV) was grown in the human A204 line. All viruses were concentrated from culture supernatants as described (3, 5).

Tissues. Baboon tissues (*Papio cynocephalus*) were obtained from Dr. J. Melnick of the Baylor College of Medicine (Houston, Texas); tissues from a related species of baboon (*Papio hamadryas*) were from Dr. H. Rabin (Litton Bionetics, Bethesda, Md.). Patas (*Erythrocebus patas*), rhesus (*Macaca mulatta*), and stumptail (*Macaca arctoides*) monkey tissues were from Drs. S. Rangan and P. Gerone of the Delta Regional Primate Research Center (Covington, La.). African green monkey tissues (*Cercopithecus aethiops*) were from Flow Laboratories (Rockville, Md.). A rhesus osteosarcoma was supplied by Dr. T. Kawakami of the University of California (Davis, Calif.), and a rhesus ovarian carcinoma, by Dr. H. Rabin. All other tissues were from Pel-Freez Biologicals, Inc. (Rogers, Ark.).

Nucleic Acid Hybridization. [^{3}H]DNA viral probes (specific activity 1.5×10^7 cpm/μg) were synthesized as described (20). The baboon and woolly monkey [^{3}H]DNA probes contained 75% and 50% of the baboon and woolly monkey 70S viral RNA sequences, respectively, at a DNA:RNA molar ratio of 1.5 (5).

[^{3}H]DNA·DNA hybridization reactions were performed as described (5). [^{3}H]DNA·RNA hybridizations were carried out at 65° in reaction mixtures containing 10 mM Tris·HCl (pH 7.4), 0.40 M NaCl, 2 mM EDTA, 0.05% (w/v) sodium dodecyl sulfate, and 20,000 cpm (1.3 ng) of [^{3}H]DNA per ml. The ratio of cellular DNA or RNA to [^{3}H]DNA varied from 1.5×10^6 to 4×10^6. Hybrids were detected with the single-strand-specific nuclease, S1 (5). [^{3}H]DNA·RNA hybridization data are plotted in Fig. 1 as a function of C_rt (21) and are corrected to a monovalent cation concentration of 0.18 M

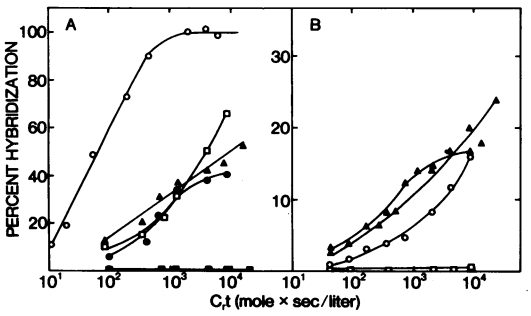

FIG. 1. (*A*) Expression of baboon type C viral information in various baboon tissues. ○, Cytoplasmic RNA extracted from canine cells infected with baboon virus, M7; □, RNA from *P. cynocephalus* liver; △, RNA from *P. cynocephalus* lung; ●, RNA from *P. hamadryas* lung; ■, RNA from rat liver. (*B*) Expression of type C baboon-related viral information in various Old World monkey tissues. △, RNA from African green monkey liver; ○, RNA from stumptail spleen; ▲, RNA from rhesus ovarian carcinoma; □, RNA from pig liver.

(22). Cellular DNA and cytoplasmic RNA were extracted as described (5).

Radioimmunoassays for p30 Proteins. The major structural proteins of the M7, RD-114, R-MuLV, and SSAV type C viruses were purified to apparent homogeneity (3), and 10-μg samples were iodinated (23) to specific activities of 5–10 μCi/μg. Competitive radioimmunoassays (24, 25, 3) and determination of radioactivity (3) were as described. Immune complexes were precipitated with antibodies directed against rabbit 7S globulin prepared in goats (Meloy Laboratories, Springfield, Va.).

Preparation of Competing Antigens. Ether extracts of cells infected with virus were prepared as described (4). Tissues (5 g wet weight) were homogenized in 20 ml of 50 mM Tris·HCl buffer (pH 8.0) containing 0.4 M KCl, 5 mM Na acetate, and 0.5% (w/v) Triton X-100. Nuclei and cell debris were sedimented (3000 × *g* for 10 min), and Na deoxycholate was added to a final concentration of 0.5% (w/v). Supernatants were extracted twice with 2 volumes of ether, the ether was evaporated, and the aqueous layer was centrifuged at 10,000 × *g* for 15 min. Supernatants were dialyzed for 18 hr against 5 mM Tris·HCl buffer (pH 7.8) containing 0.005% Triton X-100, lyophilized to dryness, resuspended in 2–4 ml of distilled water, and reclarified by centrifugation. Tissue extracts (30–60 mg of protein) were chromatographed on a 90 × 1.5-cm column of Sephadex G-100 and eluted with 50 mM Tris·HCl (pH 7.8), 0.3 M KCl, 20% glycerol, 0.01% Triton X-100, and 1 mM dithiothreitol. The column had been calibrated with dextran blue 2000, bovine serum albumin, chymotrypsinogen, and ribonuclease A. Fractions eluting between 15,000 and 50,000 molecular weight were pooled, concentrated, and dialyzed against 50 mM phosphate buffer (pH 7.5) containing 0.01% Triton X-100. Quantitation of proteins was performed as described (26).

RESULTS

Table 1 shows that various tissues from two species of baboons (*P. cynocephalus* and *P. hamadryas*) contain DNA sequences that hybridize to 72% and 50% of the M7 [^{3}H]DNA probe, respectively; patas, African green, and two species of macaques (stumptail and rhesus) contain DNA sequences that hybridize to 30%, 22%, and 20% of the baboon [^{3}H]DNA probe, respectively. Among several nonprimate tissue DNAs examined, none hybridizes to more than 2% of the probe, with the exception of normal domestic cat DNA (see ref. 5).

TABLE 1. *Detection and expression of baboon and woolly monkey type C viral information in various tissues*

		% Hybridization to cell DNA† [^{3}H]DNA probe		% Hybridization to cell RNA‡ [^{3}H]DNA probe	
		M7	SSAV	M7	SSAV
*Virus-producing cells**					
Baboon (M7)		72.0	4.0	98.0	2.0
Woolly (SSAV)		1.5	68.0	2.1	93.0
Mouse (S2CL3)		1.5	19.0	1.5	22.0
Primate tissues§					
Baboon	(*P. cynocephalus*)				
	Liver no. 1	71.0		10.0	
	Liver no. 2	72.0		>65.0	
	Lung no. 2	71.0	4.0	>50.0	5.0
	Spleen no. 2	70.0		>40.0	
	Testis no. 3	72.0		>58.0	
	(*P. hamadryas*)				
	Lung no. 1	49.0	4.0	>42.0	4.0
	Spleen no. 1	52.0		39.0	3.5
Patas	Liver	30.0	5.0	11.0	
Green monkey	Liver	22.0		20.0	
Stumptail	Spleen	19.0		15.6	3.5
	Liver	20.0		16.6	
Rhesus	Placenta	18.0	4.5	17.4	1.0
	Ovarian carcinoma	21.0	5.0	17.2	3.0
	Osteosarcoma	19.0	4.8		
Human	Spleen	4.8	5.0	2.8	3.4
Nonprimate tissues					
Pig	Liver	1.5		0.3	1.3
Rat	Liver	1.5		0.5	
Calf	Thymus	2.0	4.4		
Cat	Liver	20.5	4.5		
Mouse	Liver	2.2	19.0		

* The baboon (M7) virus was grown in a canine thymus cell line (FCf2Th), the woolly type C virus in a human rhabdomyosarcoma line (A204), and the mouse virus (S2CL3) was spontaneously released from a morphologically transformed BALB/3T3 cell line (19).

† The percentage of hybridization represents the average final saturation values to the [^{3}H]DNA probes from several experiments; all hybridizations were carried out to a C_0t of at least 10^4. Blank spaces denote hybridizations that were not tested.

‡ The percentage hybridization listed represents the average value obtained at a C_rt of 10^4.

§ DNA and RNA were extracted from various tissues. Each tissue represents a separate animal except for the baboon: one *P. hamadryas* and three *P. cynocephalus* animals were examined.

To determine whether transcription of type C viral information could be detected in Old World primate tissues, an M7 viral [^{3}H]DNA probe that had been annealed to M7 35S viral RNA was hybridized to cytoplasmic RNA extracted from various tissues. Fig. 1*A* shows that cytoplasmic RNA extracted from an M7 infected cell line hybridizes to 100% of this [^{3}H]DNA probe. Liver and lung tissue from two species of baboons transcribe from 40 to 65% of baboon type C viral information at a C_rt of 10^4. No M7-specific information could be detected in cytoplasmic RNA extracted from rat liver. The half C_rt value cannot be determined with accuracy for any of the baboon tissues tested since saturating values were not obtained, but it is estimated that the amount of viral RNA in the baboon tissues would be less than 2% of that found in productively infected canine thymus cells.

A rhesus ovarian carcinoma, a stumptail spleen, and an African green monkey liver also transcribe significant amounts

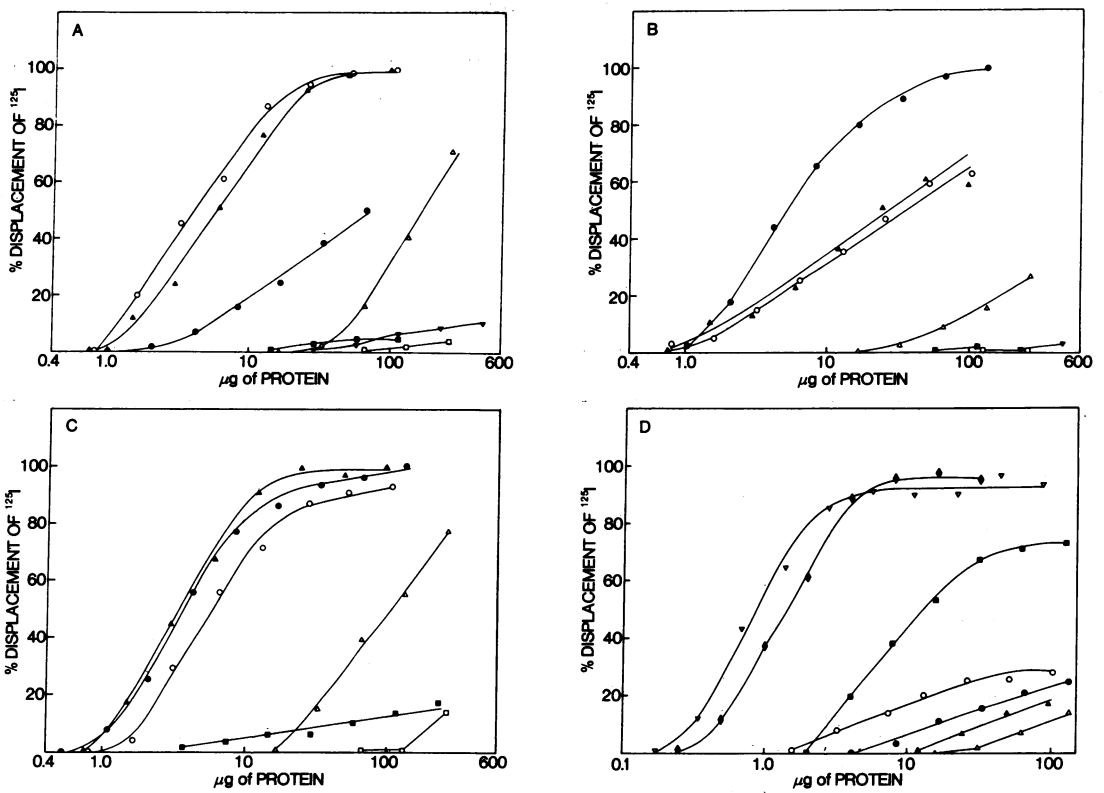

FIG. 2. Competitive radioimmunoassays for viral p30 proteins. (*A*) Species-specific assay for p30 of baboon M7 virus (anti-M7:M7). (*B*) Species-specific assay for p30 of feline RD-114 virus (anti-RD:RD). (*C*) Interspecies assay with antiserum to p30 protein of the RD-114 virus and labeled p30 protein from M7 virus (anti-RD:M7). (*D*) Interspecies assay with antiserum to p30 protein of FeLV and labeled p30 protein from R-MuLV (anti-FeLV:R-MuLV). *Competing antigens:* ○, canine cells infected with BAB8-Lg virus; ●, human M413 cells infected with RD-114 virus; ■, rat NRK cells infected with SSAV; ▼, gibbon lymphosarcoma cells producing gibbon type C virus; ▽, mouse NIH/3T3 cells infected with R-MuLV; ◆, canine cells infected with FeLV-FSV; △, baboon lung; ▲, rhesus ovarian carcinoma; □, human spleen.

of baboon type C viral related sequences (Fig. 1*B*). Although the final extent of hybridization (17–20%) is lower than that obtained with baboon tissue RNA, the relative extent of transcription is approximately the same after the data are normalized for the degree of homology (about 30%) between baboon and rhesus or African green monkey viral DNA sequences.

As shown in Table 1, primate tissues that contain baboon virus-related sequences in their DNA extensively transcribe viral-specific RNA. Variable levels of viral RNA transcription were detected in normal tissues from two species of baboons, patas, green monkey, stumptail, and rhesus, and in a rhesus ovarian tumor. A low but significant level (2.8%) of the baboon viral probe hybridized to the RNA from a normal human spleen, while background levels of hybridization (0.3–0.5%) were obtained with pig and rat tissue RNA.

A [^{3}H]DNA transcript of woolly monkey (SSAV) type C viral RNA did not hybridize significantly to the DNA of any of the primate tissues surveyed (see refs. 10 and 5). A significant level of hybridization was obtained only with normal mouse DNA, consistent with the hypothesis that SSAV most likely originated from an endogenous rodent type C virus that is infectious for primate cells (5, 27).

Since SSAV was isolated from a primate tumor tissue (8), two rhesus tumors were examined for the presence of SSAV nucleic acid sequences in their DNA and RNA. No SSAV information is present in either tumor (Table 1); less than one copy of SSAV could have been detected in the DNA of these tissues.

Given the extensive transcription of viral information in tissues of Old World monkeys related to the baboon, tissues from several primates were surveyed for detectable levels of viral p30 protein. Since the levels of p30 protein previously found in normal baboon tissues were low (1.5–3.5 ng/mg) (3), tissue extracts were chromatographed on Sephadex columns to obtain fractions enriched for viral antigen.

In an assay for the p30 protein of the M7 baboon virus (Fig. 2*A*), an extract of cells infected with the baboon BAB8-Lg virus competed efficiently for the ^{125}I-labeled baboon p30 protein. A 20% displacement of the labeled test antigen, corresponding to 2.5 ng of baboon viral p30 protein, was obtained with 1.6 μg of competing cellular protein. Thus, levels of p30 antigen in these infected cells were greater than 1500 ng/mg of protein. An extract of baboon lung tissue also competed for the labeled test antigen at much higher levels of competing protein. Based on a 15-fold protein purification obtained by gel filtration and the absence of detectable antigen activity in other column fractions, we estimate that there are 2.4 ng of p30 antigen per mg of tissue protein, corresponding to less than 0.2% of the amount of p30 protein found in exogenously infected cells.

While no p30 antigen could be detected in partially purified extracts of five tissues from Old World monkeys, a rhesus ovarian carcinoma, shown above to be transcribing viral-specific RNA, contained relatively high levels of p30 antigen (Fig. 2*A*). A 20% displacement of the labeled test antigen was obtained with 2.2 μg of competing protein, and the slope of the competition curve was indistinguishable from

the slopes obtained with the baboon lung extract or with cells infected with baboon type C virus. Given a 37-fold purification by gel filtration, we estimate that there are 30 ng/mg of viral antigen in this tissue. By this assay no p30 antigen could be detected in extracts of cells producing SSAV or gibbon type C virus or in an extract of human spleen. Neither could p30 antigen be detected in antigen-positive baboon or rhesus tissues by species-specific radioimmunoassays for the p30 proteins of SSAV or FeLV.

In an assay for the p30 protein of the feline RD-114 virus (Fig. 2*B*), crossreactions, as evidenced by the reduced slopes of the competition curves, were observed with cells infected with the BAB8-Lg virus, and with tissue extracts from the baboon lung and rhesus ovarian tumor. A reciprocal crossreaction was observed in the M7 radioimmunoassay with human cells infected with the RD-114 virus (Fig 2*A*) (see ref. 3). Extracts of cells producing high titers of SSAV or the gibbon type C virus show no crossreactions in this assay (Fig. 2*B*).

An assay that uses antiserum to RD-114 p30 protein and radio-labeled M7 p30 protein, which detects determinants shared by baboon, rhesus, and RD-114 antigens, does not detect the p30 protein of other mammalian type C viruses (Fig. 2*C*). Baboon and rhesus p30 antigens compete with similar slopes in this assay; the RD-114 p30 antigen, while immunologically distinguishable from the baboon and rhesus antigens (Fig. 2*A* and *B*), competes as efficiently as the primate antigens. The p30 antigen of SSAV (Fig. 2*C*) and of several other type C viruses [gibbon ape type C virus, R-MuLV, porcine PK-15 virus (28), and murine AT-124 virus (11)] are not readily detected (not shown). In contrast, an interspecies assay using antiserum to FeLV and radio-labeled R-MuLV p30 protein, which detects the presence of crossreacting determinants on the p30 proteins of most mammalian type C viruses (25, 4), fails to readily detect the p30 proteins in baboon and rhesus cells or in cells infected with RD-114 (Fig. 2*D*).

The degree of crossreaction between the p30 proteins of different type C viruses and the antigens found in tissues of baboon, rhesus, and a stumptail monkey spleen are summarized in Table 2. The ratio of the slope of the competition curve obtained with a crossreacting antigen to that obtained with the homologous antigen is an index of the relative degree of crossreaction between different p30 antigens (3). Baboon, rhesus, and stumptail antigens cannot readily be distinguished from one another, and both crossreact to the same extent with the RD-114 p30 antigen. The baboon, rhesus, stumptail, and RD-114 antigens are not closely related immunologically to the p30 proteins of SSAV or the gibbon type C virus, and all share an interspecies determinant different from the major interspecies determinant ("gs 3") (29) found in other mammalian type C viruses.

TABLE 2. *Relationship between p30 antigens of different type C viruses and the antigens found in primate tissues**

	Radioimmunoassay				
	Species-specific			Interspecies	
Source of antigen	Anti-M7:M7	Anti-RD:RD	Anti-SSAV:SSAV	Anti-RD:M7	Anti-FeLV:R-MuLV
Virus-infected cells					
BAB8-Lg virus	*1.0*	0.42	<0.05	*1.0*	0.20
RD-114 virus	0.45	*1.0*	<0.05	1.2	0.18
SSAV	<0.05	<0.05	1.0	0.08	0.82
Gibbon type C virus	<0.05	<0.05	0.94	N.T.	N.T.
R-MuLV	<0.05	<0.05	0.14	<0.05	*1.0*
FeLV	N.T.	<0.05	0.05	N.T.	1.0
Tissues					
Baboon lung	1.1	0.38	<0.05	0.84	0.20
Rhesus ovarian tumor	0.95	0.46	<0.05	1.1	0.21
Stumptail spleen	1.2	0.48	0.07	1.1	0.16

* Slopes of competition curves in each of five different radioimmunoassays were computed over the linear portions of the curves. A value of 1.0 was assigned to the slope obtained with the homologous competing antigen. The ratio of the slope obtained with a crossreacting antigen to that observed in the homologous system is shown in the Table and is a relative index of crossreactivity between different p30 proteins.

N.T., not tested.

DISCUSSION

Endogenous type C virogenes have been detected in the DNA of Old World monkeys and have evolved like other cellular genes in a manner consistent with phylogenetic classifications (5, 6). Our data show that normal primate tissues from these same species extensively transcribe virogene information and, in some cases, produce detectable levels of p30 antigen. Endogenous virogene expression can, therefore, be detected even in tissues of species from which infectious type C viruses have not been isolated.

The levels of transcription and translation of viral-specific information are 0.1–1.0% of those found in virus-producing cell cultures. Since [^{3}H]DNA transcripts of baboon viral RNA were used to assay for viral-specific RNA in cells of heterologous Old World monkey species, we cannot conclude whether the viral sequences are fully transcribed. However, a comparison of results obtained by [^{3}H]DNA·DNA and [^{3}H]DNA·RNA hybridization suggests that most, and very likely all, of the virogene information is expressed. Thus, primates, like chickens, mice, and cats (30–34), not only contain endogenous virogene sequences, but express this information in their tissues.

Data previously obtained with murine tissues suggest that the quantity of viral p30 antigen is greater in tumors than in normal tissues (35, 36). Our data suggest that levels of p30 antigen in several normal baboon tissues and in a stumptail spleen are 1.5–3.5 ng/mg (see ref. 3). Five other normal stumptail and rhesus tissues surveyed had less than 1 ng/mg of p30 antigen. In contrast, a rhesus ovarian carcinoma contained 10 times more p30 protein than that found in the normal tissues. A considerably larger number of primate tumors, however, would have to be examined before we can conclude that, in primates, virogene expression is more extensive in tumors than in normal tissues.

Our studies further confirm the relationship observed between endogenous primate viruses and those of the feline RD-114/CCC group (1–5). [^{3}H]DNA transcripts of RD-114 viral RNA hybridize to both baboon and rhesus cellular DNA (5), and the p30 protein of RD-114 is partially related to the baboon, stumptail, and rhesus p30 proteins. Because of these and other relationships observed between these distinct virus groups, we have proposed that RD-114 evolved from a progenitor of the Old World monkey viruses that was horizontally transmitted to ancestors of the cat at some time after these species diverged from one another (2, 4–6).

The hybridization conditions used in these studies can readily distinguish the genomes of different classes of type C viruses (27) and can detect differences among the virogenes

of different, but related, species of Old World monkeys (5). In contrast, when the slopes of the competition curves in radioimmunoassays are used as an immunologic index of antigenic crossreactions between p30 proteins, the baboon, rhesus, and stumptail monkey p30 antigens cannot be distinguished from one another with antisera prepared to the baboon virus p30 protein. Quantitative immunologic differences have been shown to correlate well with the degree of amino-acid dissimilarity among homologous proteins of different species and with the evolutionary distance between their species of origin (37–39). Studies of mammalian lysozymes of known amino acid sequence have shown few differences in immunoreactivity between rhesus and baboon enzymes and have shown that immunologic crossreactions can be detected between lysozymes of Old World monkeys and man (39). The present findings suggest that the primary amino-acid sequences of the baboon, rhesus, and stumptail p30 proteins have not undergone extensive evolutionary modification; sensitive immunologic methods with proteins from primate type C viruses may then be useful in detecting viral antigens in tissues of primates more distantly related to the Old World monkeys.

Note added in proof. Two human tumors, an ovarian carcinoma and a lymphocytic lymphoma, have been found to contain, respectively, 8 and 18 ng/mg of primate type C viral p30 antigen (Sherr and Todaro, *Proc. Nat. Acad. Sci. USA* in press). With less stringent hybridization conditions, gene sequences related to the baboon viruses are detected in ape and human cell DNA from normal as well as tumor tissues (Benveniste and Todaro, *Proc. Nat. Acad. Sci. USA*, in press). Thus, humans, like other primates, contain and can express type C virogene-specific information.

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