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Endogenous New World primate type C viruses isolated from owl monkey (*Aotus trivirgatus*) kidney cell line

(electron microscopy/DNA·DNA hybridization/genetic transmission/tumor viruses)

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ABSTRACT **A type C virus (OMC-1) detected in a culture of owl monkey kidney cells resembled typical type C viruses morphologically, but was slightly larger than previously characterized mammalian type C viruses. OMC-1 can be transmitted to bat lung cells and cat embryo fibroblasts. The virions band at a density of 1.16 g/ml in isopycnic sucrose density gradients and contain reverse transcriptase and a 60–65S RNA genome composed of approximately 32S subunits. The reverse transcriptase is immunologically and biochemically distinct from the polymerases of other retroviruses. Radioimmunoassays directed to the interspecies antigenic determinants of the major structural proteins of other type C viruses do not detect a related antigen in OMC-1. Nucleic acid hybridization experiments using labeled viral genomic RNA or proviral cDNA transcripts to normal cellular DNA of different species show that OMC-1 is an endogenous virus with multiple virogene copies (20–50 per haploid genome) present in normal owl monkey cells and is distinct from previously isolated type C and type D viruses. Sequences related to the OMC-1 genome can be detected in other New World monkeys. Thus, similar to the Old World primates (e.g., baboons as a prototype), the New World monkeys contain endogenous type C viral genes that appear to have been transmitted in the primate germ line.**

Genetically transmitted retroviruses have been isolated from a wide variety of avian and mammalian species. In primates, three different groups of endogenous retroviridae have been identified: (*i*) type C viruses (M7/M28 group) isolated from baboons (1–4) and from a closely related species, *Theropithecus gelada* (4); (*ii*) a type D virus (PO-1-Lu) isolated from an Old World langur monkey, *Presbytis obscurus* (5, 6); and (*iii*) squirrel monkey retroviruses (SMRV), which appear to be related to the type D viruses (7). This paper describes a fourth group, the endogenous type C viruses of New World primates.

The baboon type C virus has been used as a prototype in molecular hybridization studies to detect multiple copies of endogenous viral genes in the normal cellular DNA of other species of Old World monkeys and the higher apes (8). The extent of relatedness of gene sequences in other species of Old World primates to the baboon viral genes is a function of the evolutionary distance of these species from the baboon and shows that endogenous type C viral genes have been genetically transmitted in Old World monkeys and apes for at least 30 million years (8). Viral genes in the DNA of New World monkeys do not share a detectable homology to the baboon type C viral genome, probably because of its greater evolutionary distance (50–60 million years) from the New World monkeys. Thus, to date, no New World primate species could be shown to contain endogenous type C viral genes.

We now report isolation of a type C virus, designated OMC-1, released by owl monkey cells. The virus was detected in an established line of owl monkey kidney (OMK) cells and could be transmitted to heterologous cell cultures. The new isolate is different from previously characterized type C viruses in its biochemical and antigenic properties and is distinct from other primate retroviruses. Nucleic acid hybridization studies show that OMC-1 is an endogenous type C virus present in multiple copies in the DNA of New World monkeys.

MATERIALS AND METHODS

Cells and Culture Conditions. An established line of OMK cells (OMK-210) has been maintained in culture for more than 9 years and is currently in its 311th passage (9). Chromosome analyses of these cells at different passage intervals by the trypsin G-band technique demonstrated that the cells were initiated from a female *Aotus* monkey with a "type II" karyotype and a diploid count of 54 (10). The cell line has remained hypodiploid (modal chromosome number, 51–53) and a large chromosome marker has been noted (9). OMK cell lines are the optimal permissive system for a number of nonhuman primate viruses, including *Herpesvirus* saimiri and *Herpesvirus* ateles (11).

Indicator cells used for virus transmission included the bat lung line Tb1Lu (ATCC), Kirsten sarcoma virus-transformed mink cells 64J1 (12), rat kidney cells NRK (13), the mouse cell line SC-1 (14), the fetal cat embryo cell line FEC (4), rhesus monkey lung cells DBS-FRhL-1 (15), and human rhabdomyosarcoma cells, A204 (16). All of these cells were grown in Dulbecco's modification of Eagle's medium supplemented with 10% calf serum.

Viruses. Virus particles spontaneously released from cultures of OMK cells were concentrated from culture supernatants and partially purified by isopycnic banding in sucrose (ρ = 1.16 g/ml). Bat lung cells infected with the virus from the OMK cells were cloned, and those clones producing high levels of virus were the source of OMK virus. The endogenous primate retroviruses used included baboon type C virus M28 (1), langur type D virus PO-1-Lu (5), and a retrovirus isolated from a squirrel monkey, M534 (5), which appears closely related to the SMRV isolate of Heberling and coworkers (7). Other viruses used included murine leukemia virus (Rauscher strain, R-MuLV), simian sarcoma-associated virus SSAV (17), and the endogenous cat virus RD-114 (18). For the preparation of la-

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Abbreviation: OMK, owl monkey kidney.

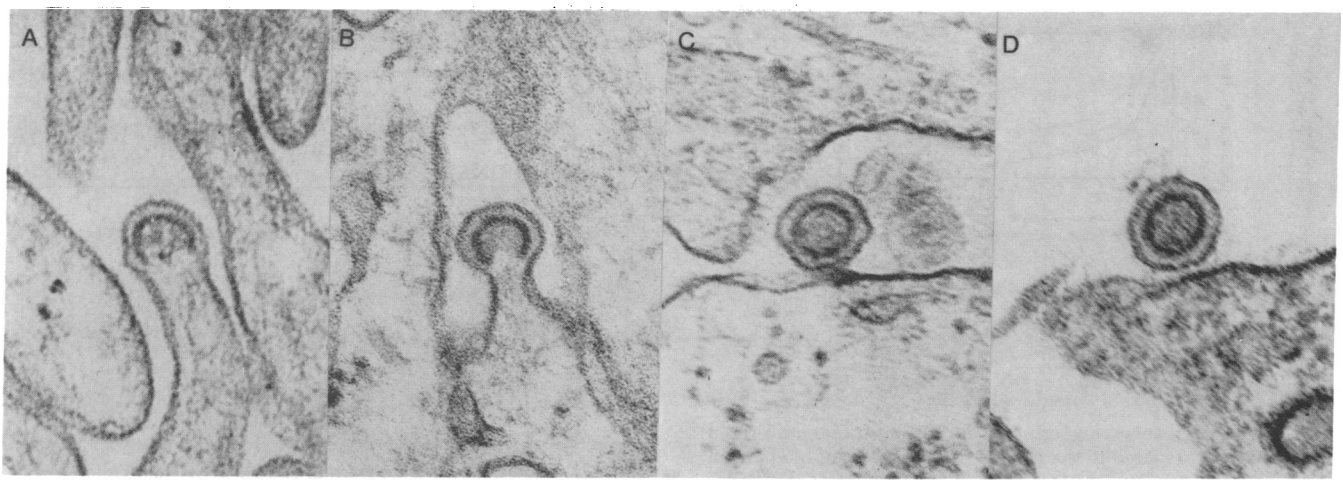

FIG. 1. Electron micrographs of OMC-1 virions from nucleoid formation under the cell membrane (*A* and *B*) to the completed, mature extracellular particle (*C* and *D*). (×100,000.)

beled DNA transcripts, supernatants from virus-producing cultures were harvested at 4-hr intervals and stored at 4° prior to concentration and partial purification of the virions (8).

Determination of Genome Size. OMK-210 cells were radiolabeled with [^{32}P]phosphoric acid, and labeled viral RNA was extracted from extracellular virions (19). The RNA was sedimented in linear 15–30% sucrose gradients (19); high molecular weight RNA was denatured in 50% formamide and electrophoresed (20) in 0.5% agarose/1.5% acrylamide composite gels together with [^{3}H]RNA standards (21). Radioactivity eluted from gel slices (22) was assayed in a Packard liquid scintillation spectrometer.

Assays for Viral Reverse Transcriptase. Polymerase assays were performed by using a polyriboadenylate template and oligodeoxythymidylate$_{12-18}$ primer (2). Divalent cation preferences were performed with various concentrations of manganese chloride or magnesium acetate (20), with disrupted virions (approximately 10 μg of protein) as the enzyme source.

Immunological Studies. Polymerase inhibition studies were performed with antisera to viral reverse transcriptases (23). Competitive radioimmunoassays for interspecies antigenic determinants of the major structural proteins of type C (23) and type D (5) viruses were performed as described.

Nucleic Acid Hybridization. [^{3}H]Thymidine-labeled DNA transcripts were prepared (6) with limiting concentrations of magnesium as divalent cation (24, 25). Labeled transcripts were resuspended in 0.2 M KOH and 5 mM EDTA, heated at 37° for 30 min, and centrifuged in 5–20% linear alkaline sucrose gradients; transcripts larger than 14 S (~5000 bases) were used in all studies. The specific activity of the [^{3}H]cDNA was 1.8×10^{7} cpm/μg. Hybridization reactions were performed at 65° in 0.75 M NaCl (26) or at 60° in 1.5 M NaCl. Hybridizations of ^{32}P-labeled viral RNA ($0.8–1.0 \times 10^{6}$ cpm/μg) to normal cellular DNAs were carried out in the presence of 50% formamide at 41°.

Labeled nonrepetitive DNA was prepared by labeling cells for 72 hr with 120 μCi of [^{3}H]thymidine per ml of culture medium. The DNA was extracted (26) and the nonrepetitive DNA was isolated by removing highly reiterated sequences that anneal by a C_0t of 200 mol·sec/liter by fractionation on hydroxyapatite (8). The specific activity of this DNA was 2.5×10^{4} cpm/μg.

The extent of DNA·DNA hybridization was determined with S_1 nuclease (27). The extent of hybridization of ^{32}P-labeled viral RNAs to cellular DNAs was determined by digestion with ribonucleases T1 and A after the hybridization reaction and then determination of the trichloroacetic acid-precipitable ^{32}P radioactivity. C_0t values (28) were corrected to a monovalent cation concentration of 0.18 M (29). DNA from cells and tissues was extracted and fragmented by ultrasound to a mean size of 6 S as determined by alkaline sucrose gradient sedimentation (26).

Electron Microscopy. Cells were fixed in gluteraldehyde, stained with uranyl acetate, and embedded in Epon as described (20, 30).

RESULTS

Late passages of cell line OMK-210 were found, by electron microscopy, to contain type C viral particles (Fig. 1). Condensation of viral nucleoids occurred at the plasma membrane, giving rise to budding type C particles with immature nucleoids; extracellular virions with central spherical nucleoids were also seen. In negatively stained preparations the virions lacked discernible spikes. The extracellular virions (1100–1200 Å) produced by OMK-210 cells were slightly larger than the baboon type C virus (~1000–1100 Å) but smaller than the type D langur virus (1200–1300 Å). Thus, by morphological criteria, the OMC-1 virus produced by OMK-210 cells is a type C virus. The virus transmitted to the bat lung cell line, Tb1Lu (see below), had an identical appearance.

Culture supernatants from OMK-210 cells were screened for virion-associated polymerase activity. With the standard conditions used for other type C or type B viruses, only low levels of synthetic activities were catalyzed by the viral enzyme (2, 20). Concentrations of KCl (0.05 M) and Triton X-100 (0.05%) were optimal. The divalent cation preferences of the OMC-1 enzyme, however, were significantly different from those of other mammalian type C viruses; 0.09 mM manganese and 3.5 mM magnesium were optimal with synthetic templates. These values are lower than those used in assays of mammalian type C viral polymerases (0.6 mM manganese, 12 mM magnesium) (20) and can be used to differentiate OMC-1 from other mammalian type C viruses (2, 5).

Fig. 2 shows that antisera prepared against the enzymes of several known type C viruses failed to inhibit the activity of OMC-1 reverse transcriptase. Antisera to the baboon virus, m7 (Fig. 2*A*), to the simian sarcoma-associated virus (SSAV) (Fig. 2*B*), and to the Rauscher murine leukemia virus (R-MuLV) (Fig. 2*C*) did not inhibit the OMC-1 enzyme. Fig. 1*D* shows

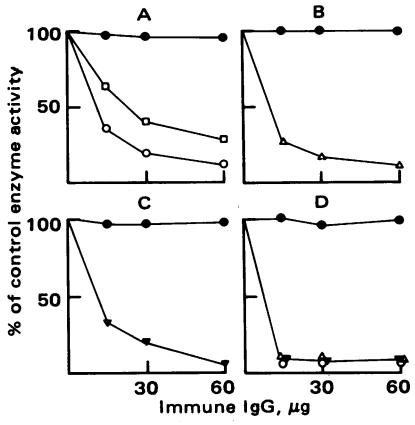

FIG. 2. Viral reverse transcriptase inhibition studies performed with rabbit IgG prepared to the reverse transcriptases of M7 baboon virus (*A*), simian sarcoma-associated virus SSAV (*B*), Rauscher murine leukemia virus R-MuLV (*C*), and endogenous cat virus, RD-114 (*D*). Values given are the percentage of [^{3}H]TMP incorporation in the presence of immune IgG relative to the incorporation with a comparable concentration of control IgG. Enzyme activity: ●, OMC-1; ○, M7; △, SSAV; ▼, R-MuLV; □, RD-114.

that a high-titer antiserum prepared against the endogenous cat RD-114 virus, which readily inhibits the polymerases of all known mammalain type C viruses tested, did not affect the activity of the OMC-1 reverse transcriptase. Negative results have also been obtained with antisera to the polymerases of avian type C viruses, the group D Mason–Pfizer monkey virus (MPMV), the langur virus (PO-1-Lu), and the squirrel monkey retrovirus M534.

OMC-1 virions were examined for viral interspecies antigenic reactivity by using competitive radioimmunoassays that detect the major structural proteins of the mammalian retroviruses. No antigenic determinants were detected in assays for type C (23) or type D (5) major viral structural proteins or in interspecies assays that detect the proteins of the squirrel monkey retroviruses (31, 32). Thus, by immunological criteria, OMC-1 is distinct from any of the previously characterized type C or type D viruses.

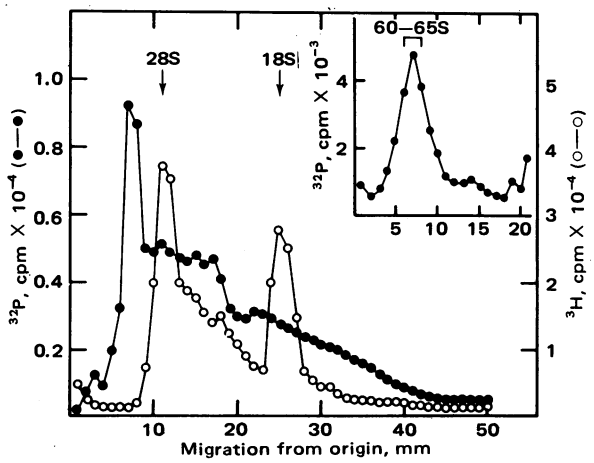

FIG. 3. Gradient centrifugation (*Inset*) and electrophoresis of denatured RNA subunits of OMC-1 RNA genome. The sedimentation coefficient of the OMC-1 RNA (60–65S) was determined by comparison with the position of 70S Rauscher murine leukemia virus RNA and 18S and 28S ribosomal RNAs run in a parallel gradient. Fractions 6–8 from the gradient were pooled, denatured with formamide, and subjected to electrophoresis on an agarose-acrylamide gel. The [^{3}H]RNA markers (18 and 28 S) were coelectrophoresed on the same gel.

Table 1. Hybridization of ^{32}P-labeled viral genomic RNAs to total cellular DNA

Cellular DNA*	% of input ^{32}P-labeled viral RNA rendered ribonuclease resistant	
	M28†	OMC-1
New World monkeys		
Owl monkey	<5.0	43.5
Woolly monkey	<5.0	7.3
Old World monkeys		
Baboon	52.3	<5.0
Rhesus	21.0	<5.0
Nonprimates		
Sheep	<5.0	<5.0
Bat	<5.0	<5.0

* DNA was extracted from normal tissues or normal cells grown in tissue culture as described (26).
† A baboon type C virus (1).

Attempts were made to transmit OMC-1 to indicator cell lines that have supported replication of other retroviridae. OMC-1 replicated only in bat TblLu cells and fetal cat embryo cells. No replication could be detected in early passages of the OMK-210 cells or various passages of an independently established OMK cell line, OMK-637.

To determine the size of the OMC-1 RNA genome, virus-producing OMK cells were labeled with [^{32}P]phosphoric acid and the RNA extracted from virions was sedimented in a sucrose gradient. A major peak of radiolabeled viral RNA sedimented at approximately 60–65S (Fig. 3 *inset*). When the high molecular weight RNA was pooled, denatured, and subjected to electrophoresis in agarose-acrylamide gels, the RNA migrated as subunits of smaller size (Fig. 3). Although much of the RNA migrated in a heterodisperse region between the 18S and 28S markers, probably as a result of nucleolytic digestion, a major radioactive species was detected in a region of the gel corresponding to molecules of approximately 32S. Thus, it appears that OMC-1 has a high molecular weight RNA genome that is dissociable into 32S subunits.

High molecular weight normal cellular DNAs from different species of New World and Old World monkeys and other nonprimate species were tested for their ability to hybridize to the radiolabeled OMC-1 viral RNA genome. ^{32}P-Labeled viral RNA from baboon type C virus M28 was also hybridized to these cellular DNAs. The data are summarized in Table 1. Owl monkey cell DNA showed the highest degree of homology with OMC-1 viral genomes; similarly, baboon (*Papio cynocephalus*) cell DNA hybridized with baboon viral RNA. The reciprocal combinations showed no hybridization above background. DNA from another New World monkey species (woolly monkey, *Lagothrix lagothricha*) hybridized to a certain extent with OMC-1 viral RNA but not with RNA from baboon virus M28. DNA from another Old World monkey (*Macaca mulatta*) hybridized with baboon viral RNA but not with OMC-1 viral RNA.

Molecular hybridizations using [^{3}H]cDNA transcripts prepared from the OMC-1 virus grown in bat lung cells showed multiple copies of type C viral genes in the cellular DNA of owl monkey tissues. Fig. 4 shows the results of an experiment in which the OMC-1 [^{3}H]DNA probe was hybridized to owl monkey liver DNA. The $C_0t_{1/2}$ for this reaction (4.5×10^1) was significantly lower than the $C_0t_{1/2}$ obtained with a nonrepetitive cellular [^{3}H]DNA probe (1.6×10^3 mol·sec/liter), indicating the presence of approximately 30–40 copies of OMC-1-related

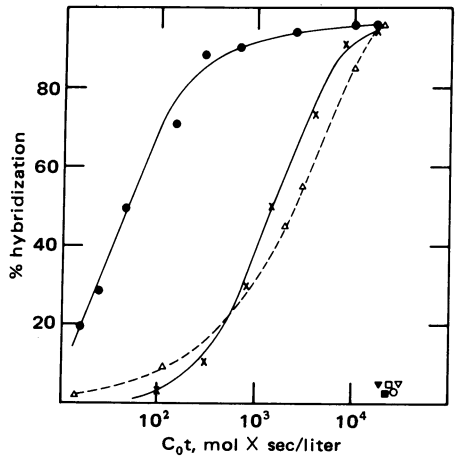

FIG. 4. Hybridization of an OMC-1 [^{3}H]DNA transcript and a nonrepetitive owl monkey cellular [^{3}H]DNA probe to the DNA of various species. OMC-1 viral transcript hybridized to the DNA of: ●, owl monkey liver; △, bat cells infected with OMC-1; ▼, woolly monkey kidney; □, howler monkey liver; ▽, spider monkey spleen; ■, human spleen; ○, rhesus liver. The hybridization of the nonrepetitive owl monkey cellular DNA probe to owl monkey liver is shown by (×).

viral genes in the DNA of owl monkeys. The cellular DNA from bat lung cells infected with OMC-1 showed a $C_0t_{1/2}$ of 1.9 × 10^3 mol·sec/liter, consistent with having acquired a single genome per cell.

With less stringent hybridization conditions than used for the experiment shown in Fig. 4 [1.5 M NaCl, 60° (8)], greater final extents were reproducibly obtained with the DNAs of woolly, howler (*Alouatta* spp.), squirrel (*Saimiri* spp.), and spider (*Ateles* spp.) monkeys (23–29%) than with DNAs from Old World primates and nonprimates (6–16% final extents) (Table 2). Multiple copies of viral genes in the normal cellular DNA of owl monkeys and related viral gene sequences in the DNA of other New World monkeys establishes that OMC-1 is an endogenous virus of New World primates.

Whereas the M28 baboon viral cDNA hybridizes preferentially to the DNA of Old World monkeys, the OMC-1 probe detects sequences only in New World monkey species. Attempts to detect related sequences in various nonprimates, including those known to have complete endogenous type C viruses (mouse, cat, pig, chicken) as well as several New World mammals (peccary, margay cat, guinea pig), did not show increased final extents of hybridization. Thus, although OMC-1 is an endogenous primate type C virus, it could not be shown to be related to previously characterized endogenous type C viral genes found in the DNA of Old World monkeys and apes. In other studies, we were unable to detect hybridization between the OMC-1 probe and the RNA of the other primate retroviruses, the endogenous virus of langurs and that of squirrel monkey; correspondingly, probes prepared from the genomic RNA of those as well as probes from various murine, feline, avian, and porcine type C viruses did not hybridize to OMC-1 viral RNA. Thus, the virus isolate described here is different from all previously described retroviruses, including others that are also endogenous in primate cellular DNA.

DISCUSSION

The OMC-1 virogene-related sequences present in cellular DNAs of various other New World primates suggest that they, like the Old World primates, contain genetically transmitted viral genes that have been transmitted through the germ line for several million years.

Table 2. Hybridization of owl monkey and baboon viral DNA transcripts to cellular DNA of various species

Species*	% hybrid with [^{3}H]viral DNA transcript† OMC-1
Owl monkey cell culture	95.9
Owl monkey tissue	88.9
Other New World Primates	
Spider monkey	26.2
Howler monkey	29.2
Woolly monkey	23.3
Squirrel monkey	23.6
Old World Primates	
Baboon	14.1
Rhesus	12.9
Langur	15.6
Human	13.8
Nonprimates	
Mouse	10.9
Cow	15.0
Sheep	12.2
Cat	14.3
Margay cat	15.9
Pig	12.1
Peccary	11.2
Bat	13.7
Guinea pig	8.9
Chicken	6.6

* DNA was extracted from normal tissues of the species shown by using published methods (26).

† Labeled DNA transcripts prepared to OMC-1 grown in bat lung cells were hybridized to primate and nonprimate cellular DNA. Hybridizations were performed at 60° in 1.5 M NaCl to $C_0t > 10^4$ mol·sec/liter.

The lack of homology between OMC-1 and the baboon type C viruses could reflect the extent of evolutionary divergence of the different primate families. Alternatively, OMC-1 may represent a distinct class of type C viruses with a more related counterpart in Old World monkeys. The failure to demonstrate antigenic relationships between OMC-1 and the baboon type C virus (or any other type C viral group) does not permit us to distinguish formally between these possibilities. A third possibility to be considered is that either or both viruses have been introduced into the primate genome after the genetic and physical separation of Old World and New World primates. If antisera prepared to OMC-1 could be shown to react with the homologous proteins of baboon viruses or vice versa, the data would suggest a common origin for both type C viral groups. Such reagents could then facilitate the design of broad immunological assays that would detect interspecies antigens shared by all primate type C viruses. A similar design has been described recently for the primate type D viruses, using antisera to the major structural protein of langur virus or Mason–Pfizer monkey virus and the squirrel monkey type D virus (31, 32).

The endogenous owl monkey type C virus is the second endogenous retrovirus isolated from New World monkeys. The squirrel monkey retrovirus (7, 31) and subsequent squirrel monkey isolates such as M534 (5) are also endogenous. It and OMC-1, however, are quite different morphologically; the squirrel monkey virus appears to be more closely related to the group D viruses isolated from Old World primates (33, 34). Thus, both Old World and New World monkeys contain at least one example of each of two distinct sets (type C and type D) of endogenous viral genes (Table 3).

Table 3. Partially characterized endogenous retroviruses of primates

Virus types	New World	Old World
Type C	OMC-1	Baboon viruses (M28, BAB-K, PP-1, etc.)
Type D	SMRV group (various isolates)	Langur virus and related isolates

OMK-210 cells and other owl monkey cell cultures have been used for propagating oncogenic herpesviruses *Herpesvirus* saimiri and *Herpesvirus* ateles. Previous reports had concluded that type C viruses or antigens related to known type C viruses could not be detected in owl monkeys bearing tumors (35, 36). Purified *Herpesvirus* saimiri DNA can produce malignant lymphomas in cotton-top marmoset monkeys (*Saguinus oedipus*) (37). The above results imply that the pathogenic strains of herpesvirus produce their oncogenic effect in primates directly. There is, at present, no evidence to suggest that the owl monkey type C virus described here had any role in the oncogenic process.

Although initial retroviral isolates were obtained primarily from birds, rodents, and cats, the various groups of primate retroviridae have been identified only recently. At present, however, there appear to be as many different classes and examples of primate retroviruses as there are different retroviral groups among the other vertebrates. The endogenous retroviral groups and the maintenance of viral genes in the germ line allow the speculation that endogenous viral genes play a role in phylogenesis or ontogenesis of higher organisms. In birds and mammals other than primates, the retroviridae have also been shown to be etiologic agents for certain forms of neoplasia and, perhaps, autoimmune diseases. Further studies may resolve whether the endogenous owl monkey type C viral genes play a role in the high incidence of apparent autoimmune diseases, hemolytic anemias, and glomerulonephritis found in owl monkeys (38, 39). The advent of the new primate retroviruses may lead to a better assessment of the physiologic or pathologic importance of endogenous viral genes in species more evolutionarily related to man.

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