Proc. Natl. Acad. Sci. USA
Vol. 74, No. 12, pp. 5734–5738, December 1977
Medical Sciences

Endogenous New World primate retrovirus: Interspecies antigenic determinants shared with the major structural protein of type-D RNA viruses of Old World monkeys

(squirrel monkey retrovirus/Mason–Pfizer monkey virus/nucleic acid hybridization)

SHIGEO HINO*, STEVEN R. TRONICK*, RICHARD L. HEBERLING†, SEYMOUR S. KALTER†, ALFRED HELLMAN*, AND STUART A. AARONSON*

*National Cancer Institute, Bethesda, Maryland 20014; and †Southwest Foundation for Research and Education, San Antonio, Texas 78284

Communicated by Robert J. Huebner, September 29, 1977

ABSTRACT **A reverse transcriptase-containing virus has recently been isolated from a squirrel monkey *(Saimiri sciureus)*. Molecular hybridization studies demonstrate that the squirrel monkey retrovirus (SMRV) is endogenous to this New World primate, yet lacks detectable nucleotide sequence homology with cellular DNAs of representative Old World primates or with the genomes of previously isolated Old World primate retroviruses. The 35,000-dalton major structural protein (p35) of SMRV was purified and shown to possess antigenic determinants distinct from those of known retroviruses. While SMRV was found to lack antigenic determinants broadly shared among mammalian type-C viruses, immunologic crossreactivity was demonstrated between SMRV p35 and the major structural protein (p26) of Mason–Pfizer monkey virus, a prototype type-D retrovirus of Old World monkeys. These findings support the concept that SMRV and Mason–Pfizer monkey virus are evolutionarily related, and raise the possibility that a progenitor of type-D retroviruses became genetically associated with primates at a very early time in their evolution.**

Reverse transcriptase-containing viruses, in the family Retroviridae (1), have been isolated from a large number of vertebrate species. Interest in retroviruses has to a large extent focused upon type-C RNA viruses, as evidence has accumulated for the involvement of these viruses in naturally occurring tumors of several species, including some primates (for a recent review, see ref. 2). However, other retroviruses, such as mouse mammary tumor virus and bovine lymphosarcoma virus, that are distinguishable from type-C viruses on the basis of their morphogenetic, biochemical, and immunologic properties, have also been implicated in neoplasia (3–6). A property of retroviruses that appears to make them unique among animal viruses is that in many instances these viruses have been shown to be transmitted within the germ line of a species (2). Endogenous retroviruses have been demonstrated by a variety of techniques, including spontaneous or chemical induction from virus-negative cells (7–9), detection of nucleotide sequence homology between cellular DNA of a species and the retrovirus genome (10–14), and demonstration of sub-viral expression in virus-negative tissues by sensitive immunologic techniques (15–18).

The widespread distribution of endogenous retroviruses among mammalian species has provided impetus to the search for analogous viruses of humans. To date, endogenous retroviruses of primates include type-C viruses (19, 20) and type-D viruses (21–24) of Old World monkeys. Recently, a retrovirus has been isolated from a New World primate, the squirrel monkey *(Saimiri sciureus)* (25). In the present report, the squirrel monkey retrovirus has been characterized biochemically and immunologically in an effort to elucidate its origin and determine its genetic relationship to known primate retroviruses.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

METHODS

Viruses and Cells. The squirrel monkey retrovirus (SMRV) was propagated in canine fetal thymus (CFT), human (A204), or rhesus fetal lung cells (FrhL-1) as previously reported (25). Other viruses were grown in this laboratory or obtained through the Office of Resources and Logistics, National Cancer Program, National Cancer Institute, Bethesda, MD. Type-C viruses included endogenous viruses of the baboon *(Papio cynocephalus)* (19) and deer *(Odocoileus hemionus)* (14), and viruses isolated from the woolly monkey *(Lagothrix* sp.) (26) and the gibbon *(Hylobates lar)* (27). Other retroviruses studied were Mason–Pfizer monkey virus (MPMV) (21, 22), bovine lymphosarcoma virus (BLV) (5), mouse mammary tumor virus (MMTV) (3, 28), and simian syncytium-forming virus (29).

Molecular Hybridization. Viral [^{3}H]cDNA was prepared from SMRV grown in CFT cells. Reaction mixtures contained 20 mM Tris·HCl, pH 7.8; 40 mM KCl; 1 mm dithiothreitol; 10 mM $MgCl_2$; 0.4 mM each dATP, dCTP, and dGTP; 0.01 mM [^{3}H]dTTP (50 Ci/mmol, New England Nuclear); actinomycin D (Calbiochem) at 75 μg/ml; 0.015% (vol/vol) Triton X-100; and viral protein at 50 μg/ml. ^{3}H-Labeled viral cDNA was purified as previously described (30).

Cellular and viral RNA were isolated according to previously reported methods (30). Cellular DNA was purified as described by Britten *et al.* (31) and fragmented to an average size of 450 nucleotides using a Virtis model 60K homogenizer.

Conditions for DNA·DNA hybridization and DNA·RNA hybridization have been described in detail (14). Hybrid formation was assayed by the S_1 nuclease method (32, 33). Hybridization data, expressed in terms of C_0t and C_rt (moles of nucleotide × seconds × liters^{-1}) for DNA·DNA and RNA·DNA reactions, respectively, were corrected to a monovalent cation concentration of 0.18 M (31).

Labeling of Virions with Radioactive Precursors. Exponentially growing CFT cells producing SMRV were washed twice with phosphate-buffered saline and labeled for 16 hr with 50 μCi/ml of [^{3}H]leucine (42 Ci/mmol) or [^{3}H]glucosamine (9 Ci/mmol) (New England Nuclear). Culture fluids were clarified by centrifugation at 3000 × *g* for 10 min, and virions

Abbreviations: SMRV, squirrel monkey retrovirus; MPMV, Mason–Pfizer monkey virus; BLV, bovine lymphosarcoma virus; MMTV, mouse mammary tumor virus; $NaDodSO_4$, sodium dodecyl sulfate; C_0t and C_rt, product of concentration and incubation time in DNA·DNA and RNA·DNA hybridization reactions (moles of nucleotide × sec × liters^{-1}).

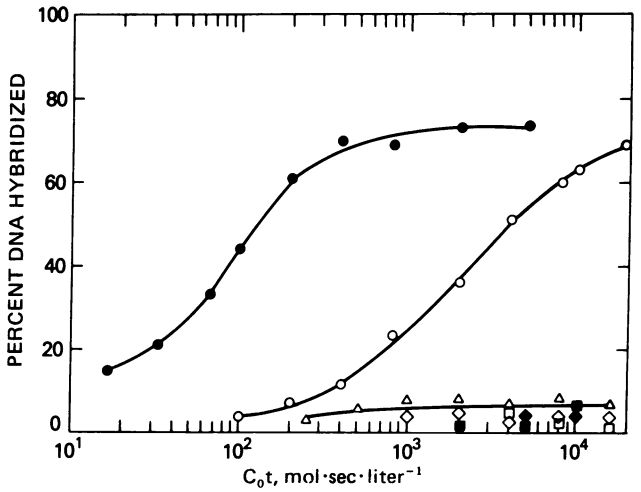

FIG. 1. Nucleotide sequence homology of SMRV cDNA with cellular DNAs of New and Old World primates. [^{3}H]cDNA prepared from SMRV was hybridized with DNA isolated from livers of squirrel monkey *(Saimiri sciureus)* (●), capuchin *(Cebus capucinus)* (△), rhesus monkey *(Macaca mulatta)* (♦), baboon *(Papio papio)* (◇), chimpanzee *(Pan troglodytes)* (■), and dog *(Canis familiaris)* (□). The kinetics of the hybridization of squirrel monkey unique-sequence cell [^{3}H]DNA with a 1000-fold excess of squirrel monkey cellular DNA are also shown (○). The results represent mean values of at least two experiments.

were purified by successive discontinuous and linear sucrose gradient centrifugations.

Purification of the Major Structural Protein of SMRV. Viral protein was purified as described previously (34). Briefly, virions were disrupted with 1.0% Triton X-100, pH 9.0, and centrifuged at 100,000 × *g* for 60 min. The supernatant was dialyzed against 0.01 M *N,N*-bis(2-hydroxyethyl-2-aminoethanesulfonic acid (Bes), pH 6.5/0.001 M EDTA/0.1% Triton X-100, and applied to a 1.0- × 5-cm phosphocellulose column (P11, Whatman). Proteins were eluted with a 100-ml 0–1 M KCl linear gradient in the same buffer. A 50-μl aliquot of every third fraction (1.5 ml) was subjected to 12.5% $NaDodSO_4$/polyacrylamide gel electrophoresis to locate the major virion protein peak. Peak fractions (0.4–0.5 M in KCl) were then pooled, dialyzed, lyophilized, and chromatographed on an agarose gel column (Bio-Rad A-5 m) in the presence of 6 M guanidine·HCl (35). Each fraction (2 ml) was dialyzed against 0.01 M Tris, pH 7.8/0.01 M NaCl/0.001 M EDTA/0.1% Triton.

Radioimmunoassay Procedures. Purified viral proteins were radioiodinated with ^{125}I by the chloramine T method (36) to specific activities of 20–50 μCi/μg, and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Methods for radioimmunoassays by the double antibody technique have been described previously (34). Rabbit and goat antisera against sucrose-gradient banded SMRV were prepared at the Southwest Foundation and the National Cancer Institute, respectively. Each was elicited by sequential weekly immunizations with approximately 10^{11}–10^{12} detergent-disrupted SMRV particles. Other antisera were produced by Huntingdon Laboratories under a contract with the Virus Cancer Program, National Cancer Institute.

RESULTS

Nucleotide Sequence Homology between SMRV and Squirrel Monkey Cellular DNA. The endogenous nature of a retrovirus can be determined by the demonstration of extensive base sequence homology between the viral genome and the DNA of uninfected cells of a particular species. Therefore, to determine whether SMRV was endogenous to the squirrel monkey, a [^{3}H]cDNA transcript of the SMRV genome was prepared. As shown in Fig. 1, SMRV [^{3}H]cDNA hybridized extensively (75%) with normal squirrel monkey DNA at the highest C_0t value tested (2×10^4). The t_m (melting temperature) of the hybrids was 89°, indicating a high degree of correct base-pairing. Significant reactions were not observed with the DNAs of another New World monkey (capuchin) or several Old World primates (rhesus monkey, baboon, and chimpanzee). The $C_0t_{1/2}$ value for the hybridization reaction between the SMRV probe and squirrel monkey DNA was 70, whereas the $C_0t_{1/2}$ for the reassociation of unique-sequence squirrel monkey DNA was about 2×10^3. These results indicate the presence of about 30 copies of the SMRV viral genome per haploid cell.

Table 1. Analysis of nucleotide sequence homology in retroviruses isolated from primates

Source of RNA	Maximum hybridization (%) with cDNA of:* SMRV	MPMV	Baboon endogenous virus	Woolly monkey virus
SMRV	82	<3	<3	<3
MPMV	<3	83	<3	<3
Baboon endogenous virus	<3	<3	95	<3
Woolly monkey virus	<3	<3	<3	93
Gibbon leukemia virus	<3	<3	<3	55

* RNA·cDNA hybridizations were performed as described in *Methods*. Reactions with heterologous viral RNAs were carried out to C_rt values 10- to 100-fold in excess of those required for maximum hybridization with the homologous viral RNA.

Analysis of SMRV for Nucleotide Sequence Homology with Other Retroviruses. The experiments described above, as well as recent findings utilizing viral RNA as a probe (37), established SMRV as an endogenous virus of the squirrel monkey. In order to test whether SMRV was genetically related to other retroviruses, SMRV [^{3}H]cDNA was first examined for its ability to hybridize with the RNAs of representative primate retroviruses. As shown in Table 1, SMRV RNA annealed 82% of the probe; however, RNAs of other retroviruses tested, including MPMV and type-C viruses of New and Old World primates, did not react significantly. Moreover, ^{3}H-labeled cDNAs prepared from these same viruses did not react with SMRV RNA, although each was annealed to an extent of at least 80% by its respective homologous RNA.

Radioimmunoassay for the Major Structural Protein of SMRV. The lack of detectable nucleotide sequence homology between viral genomes (Table 1) does not exclude their genetic relatedness, because certain proteins of all known mammalian type-C viruses share common antigenic determinants (38–40). Thus, in a further effort to demonstrate genetic relatedness of SMRV with other retroviruses, immunological studies were performed. The structural proteins of SMRV were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis using [^{3}H]-leucine-labeled virus. Five major radioactive peaks with apparent molecular weights of 85,000, 35,000, 20,000, 14,000, and 9000 were observed. The 35,000-dalton protein incorporated the largest amount of radioactivity, suggesting that it was the major virion component. [^{3}H]Glucosamine was incorporated only into a 85,000-dalton peak, indicating that the 85,000-dalton protein was a glycoprotein (Fig. 2). On the basis of these results, SMRV proteins were designated gp85, p35, p20, p14, and p9.

The major protein of SMRV was isolated by sequential

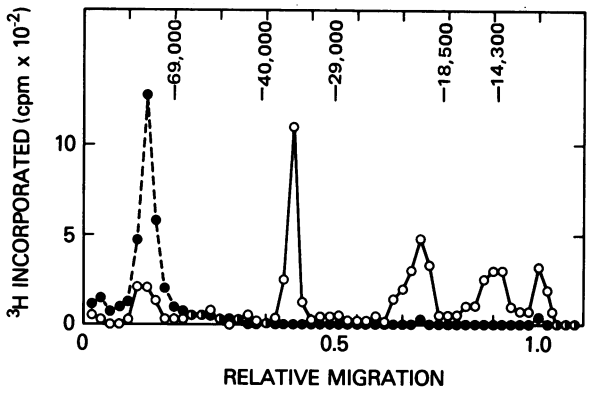

FIG. 2. NaDodSO$_4$/polyacrylamide gel electrophoretic analysis of SMRV proteins. SMRV, labeled with [^{3}H]leucine (○) or [^{3}H]glucosamine (●), was subjected to 12.5% polyacrylamide gel electrophoresis in the presence of 0.1% NaDodSO$_4$. Molecular weight markers included bovine serum albumin (69,000), aldolase (40,000), carbonic anhydrase (29,000), β-lactoglobulin (18,500), and lysozyme (14,300).

phosphocellulose and agarose gel filtration chromatography as described in *Methods*. In each case, the peak containing p35 was identified by NaDodSO$_4$/polyacrylamide gel electrophoretic analysis of aliquots of individual column fractions. Following the two purification steps, the 35,000-dalton protein was estimated to be at least 90% pure when analyzed by NaDodSO$_4$ gel electrophoresis. SMRV p35, radioiodinated by the chloramine T method, migrated as a single sharp peak with an apparent molecular weight of 35,000 on NaDodSO$_4$ gels (Fig. 3).

To develop an immunoassay for SMRV p35, rabbit antiserum prepared against SMRV was used to precipitate SMRV ^{125}I-labeled p35 (^{125}I-p35). In a homologous competition immunoassay, utilizing limiting antiserum to SMRV to precipitate SMRV ^{125}I-p35, detergent-disrupted SMRV competed fully, requiring less than 1 ng of viral protein for detection. In contrast, none of several representative mammalian type-C viruses reacted in this assay, nor did MPMV, MMTV, BLV, or simian syncytium-forming virus (Fig. 4*A*). The virus-coded nature of a protein is strongly implied by the preservation of its antigenic determinants following growth in cells of diverse species. As shown in Fig. 4*A*, SMRV, grown in canine, human, or rhesus monkey cells reacted identically, indicating that p35 was coded for by the SMRV genome. In a broadly reactive immunoassay

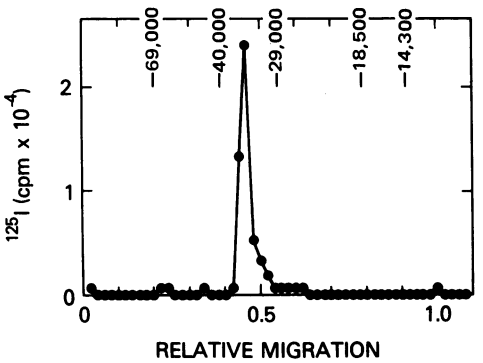

FIG. 3. NaDodSO$_4$/polyacrylamide gel electrophoretic analysis of ^{125}I-labeled SMRV p35. SMRV p35 was isolated by successive phosphocellulose and Bio-Gel A-5m agarose gel chromatography in the presence of 6 M guanidine hydrochloride. The protein was labeled with ^{125}I and subjected to 12.5% polyacrylamide gel electrophoresis in the presence of 0.1% NaDodSO$_4$. Molecular weight markers are as indicated in the legend to Fig. 2.

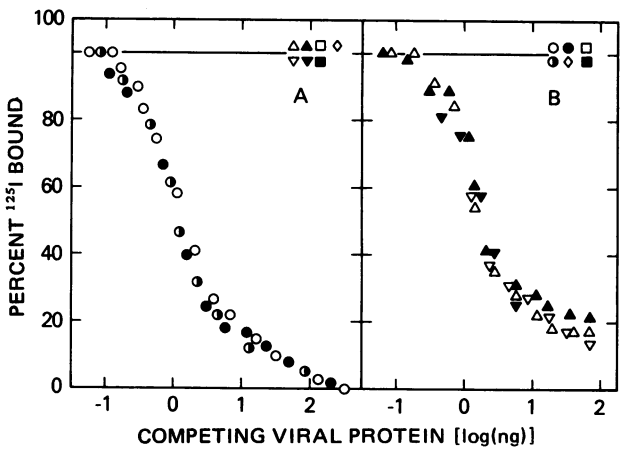

FIG. 4. Unique antigenic determinants of SMRV p35. SMRV and other representative retroviruses were tested for their ability to compete in radioimmunoassays for (*A*) SMRV p35, utilizing limiting anti-SMRV serum (1/60,000) to precipitate ^{125}I-labeled SMRV p35 and (*B*) mammalian type-C viral p30, utilizing limiting anti-RD114 (1/40,000) to precipitate ^{125}I-labeled p30 of Rauscher murine leukemia virus. Assays were performed as described in *Methods*. Detergent-disrupted virions served as the sources of antigen in each assay. SMRV, grown in canine fetal thymus (CFT) (○), rhesus (FrhL-1) (◑), and human (A204) (●) cell lines; woolly monkey virus (△), gibbon leukemia virus (▽), deer virus (▲), baboon endogenous virus (▼); MPMV grown in rhesus (FrhL-1) (□) and human (A204) (■) cell lines; MMTV, BLV, and simian syncytium-forming virus (◇).

capable of detecting p30s of known mammalian type-C viruses (41), SMRV failed to react, while each type-C virus competed completely (Fig. 4*B*). Moreover, in radioimmunoassays for the major proteins of MPMV (42), BLV (43), and MMTV (44), SMRV showed no detectable crossreactivity (data not shown). These results establish that SMRV is immunologically distinct from known mammalian retroviruses.

Demonstration of Antigenic Relatedness between the Major Internal Proteins of SMRV and MPMV. In further attempts to detect antigenic homology between SMRV and other viruses, the binding of SMRV ^{125}I-p35 by antisera raised against representative retroviruses was examined. As shown in Table 2, high-titered antiserum directed against MPMV bound SMRV ^{125}I-p35 at a titer of 10,000. The binding was 61% at serum dilution of 1/80, comparable to the maximum binding

Table 2. Immunoprecipitation of the SMRV major structural protein (p35) by antisera prepared against mammalian retroviruses

	Antibody titer against ^{125}I-labeled major internal protein of:*		
Antisera against	SMRV	MPMV	Homologous virus
SMRV	150,000	2,400	—
MPMV	10,000	1,200,000	—
Woolly monkey virus	<80	<80	500,000
Feline leukemia virus	<80	<80	1,000,000
Rauscher murine leukemia virus	<80	<80	500,000
RD114	<80	<80	500,000
Baboon virus	<80	<80	300,000
BLV	<80	<80	10,000
MMTV	<80	<80	60,000

* Titers are expressed as the reciprocal of the highest serum dilution capable of binding 10% of the appropriate ^{125}I-labeled probe (10,000 cpm).

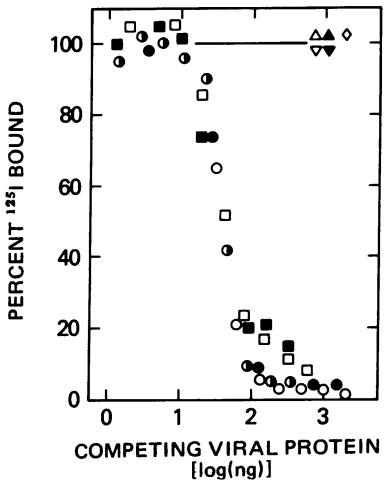

FIG. 5. Demonstration of interspecies antigenic determinants shared by SMRV and MPMV. SMRV and other representative retroviruses were tested for their ability to compete in a radioimmunoassay utilizing limiting anti-SMRV (1/600) to precipitate MPMV ^{125}I-p26. Symbols are the same as those used in Fig. 4.

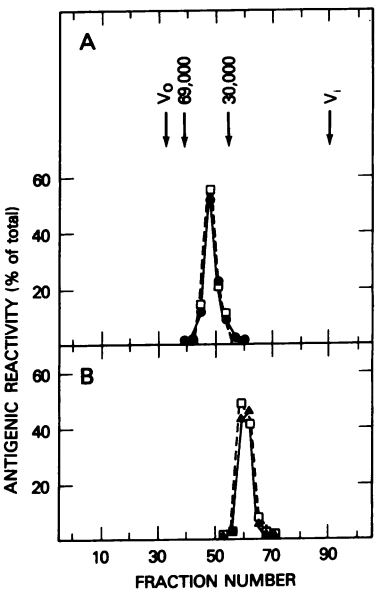

FIG. 6. Molecular size analysis of unique and shared antigenic determinants of SMRV and MPMV. Two milligrams each of SMRV (*A*) or MPMV (*B*) was disrupted by incubation with 0.5% Triton X-100 in a solution containing 0.01 M Tris·HCl buffer, pH 7.8/0.25 M NaCl/0.001 M EDTA for 60 min at 37°. Following centrifugation at 100,000 × *g* for 60 min, supernatants were applied to AcA 44 Ultrogel columns. Fractions (1 ml) were tested in competition radioimmunoassays utilizing anti-SMRV serum to bind either SMRV ^{125}I-p35 (●) or MPMV ^{125}I-p26 (□) and in an immunoassay utilizing anti-MPMV serum to bind MPMV ^{125}I-p26 (▲). The arrows indicate the elution positions of blue dextran (V_0), bovine serum albumin (69,000 daltons), murine leukemia virus ^{125}I-p30, and ^{125}I- (V_i).

achieved with anti-SMRV serum (81%). Reciprocally, antiserum to SMRV precipitated MPMV ^{125}I-p26 at a titer of 2400 (Table 2); the binding was 57% at serum dilution of 1/80, compared to 85% achieved with the homologous antiserum. In contrast, there was no detectable precipitation of either iodinated probe by high-titered antisera prepared against other retroviruses.

The binding of SMRV ^{125}I-p35 and MPMV ^{125}I-p26 by antisera prepared against either virus strongly suggested the presence of common antigenic determinant(s) on these major internal proteins. The specificity of these reactions was demonstrated in a competition immunoassay utilizing limiting anti-SMRV serum to precipitate MPMV ^{125}I-p26. As shown in Fig. 5, both SMRV and MPMV competed completely and with almost identical efficiency. In contrast, no reactivity of any other retrovirus was detected. Analogous results were obtained in the reciprocal assay, utilizing anti-MPMV to bind SMRV ^{125}I-p35 (data not shown). To conclusively identify the virion proteins responsible for the immunologic crossreactivity between SMRV and MPMV, detergent-disrupted viruses were subjected to gel filtration chromatography according to methods previously described (39). In each case, the antigenic reactivity detected in either of the interspecies immunoassays cochromatographed with the respective major structural protein, as determined by both molecular size and antigenic reactivity in the appropriate homologous immunoassay (Fig. 6). These findings establish that the major internal proteins of SMRV and MPMV share interspecies antigenic determinants.

DISCUSSION

In the present report, the origin of a recently isolated retrovirus of a New World primate and its genetic relatedness to known primate retroviruses have been determined. By molecular hybridization, SMRV demonstrated no detectable nucleotide sequence homology with representative type-C and type-D viruses of Old World primates. Its major structural protein was shown to have a molecular weight of 35,000, higher than that of analogous proteins of known mammalian retroviruses. Moreover, SMRV p35 possessed antigenic determinants distinct from those of any other known retrovirus, firmly establishing it as a new virus isolate.

Since the initial discoveries of their genetic transmission, endogenous retroviruses have been isolated from a wide variety of species (for review, see ref. 2). In many additional species, that have not yielded complete infectious virus, the presence of endogenous viral information has been implied by demonstration of virus-related nucleotide sequences and/or detection of subviral expression of endogenous viral proteins. In the present report, SMRV was demonstrated to share extensive nucleotide sequence homology with the genome of the squirrel monkey, indicating its origin as an endogenous virus of that species. Similar findings have recently been reported utilizing labeled SMRV RNA as a probe (37). In the present studies, DNA reassociation kinetics indicated the existence of multiple (more than 10) SMRV copies within the squirrel monkey cellular genome. In contrast, there was no demonstrable hybridization of the SMRV cDNA probe with DNAs of several representative New and Old World primates. Thus, if viruses analogous to SMRV are genetically transmitted within these other primates, the genomes of such viruses must have diverged sufficiently so as to be no longer detectable with SMRV as a molecular probe.

Previous studies have shown that several type-C viral proteins share antigenic determinants with analogous proteins of all known mammalian type-C viruses. These findings have provided strong support for the concept that mammalian type-C viruses share a common evolutionary progenitor (39). The failure of SMRV to compete in a broadly reactive immunoassay that detects the p30 proteins of known mammalian type-C viruses establishes SMRV as distinct from this retrovirus group. Instead, the demonstration of specific binding of ^{125}I-labeled major proteins of SMRV or MPMV by antiserum directed against the reciprocal virus provides strong evidence of the immunologic and, thus, genetic relatedness of SMRV and MPMV, a prototype type-D retrovirus.

The demonstration that SMRV is endogenous to a New World primate and yet is genetically related to type-D viruses of Old World monkeys must be considered in light of recent evidence concerning the origin of MPMV-like viruses. Drohan and coworkers have reported the presence of MPMV-related information within cellular DNAs of many Old World monkeys (24). Moreover, a new MPMV-like isolate of the langur, another Old World monkey, appears to show even more extensive nucleotide sequence homology with its species of origin (23). These findings, taken together, have implications concerning the search for retroviruses of higher primates. If type-D viruses are genetically transmitted in representatives of both New and Old World primates, it is possible that a progenitor type-D virus became established within the genome of a common primate ancestor, prior to the divergence of New and Old World monkeys, some 50–60 million years ago (45). Alternatively, separate infection leading to stable integration of the viral genome may have occurred in progenitors of these major primate lines following their divergence. If such events occurred prior to the very long geographical separation of New and Old World monkeys, it is likely that endogenous type-D viruses may be very widespread among living representatives of most, if not all, primate species. The presently described interspecies radioimmunoassay, capable of detecting shared antigens of primate type-D viruses may, thus, be useful in the further search for evidence of endogenous retrovirus expression in man.

(Following submission of this manuscript, we became aware of an independent study from another laboratory providing similar evidence [see accompanying article (46)].)

The authors thank Drs. M. Barbacid and J. M. Krakower for helpful discussions, and C. Winters, P. Williams, and B. McGlaughlin for excellent technical assistance. This work was supported in part by Contract N01-CP-61024 from the Virus Cancer Program of the National Cancer Institute.

1. Fenner, F. (1976) *Virology* **71,** 371–378.
2. Aaronson, S. A. & Stephenson, J. R. (1976) *Biochim. Biophys. Acta* **458,** 323–354.
3. Bittner, J. J. (1942) *Science* **95,** 462–464.
4. Gross, L. (1970) *Oncogenic Viruses* (Pergamon Press, New York and London).
5. Olson, C., Miller, L. D., Miller, J. M. & Hoss, H. E. (1972) *J. Natl. Cancer Inst.* **49,** 1463–1467.
6. Gilden, R. V., Long, C. W., Hanson, M., Toni, R., Charman, N. P. Oroszlan, S., Miller, J. M. & Van der Maaten, M. J. (1975) *J. Gen. Virol.* **29,** 305–314.
7. Aaronson, S. A., Hartley, J. W. & Todaro, G. J. (1969) *Proc. Natl. Acad. Sci. USA* **64,** 87–94.
8. Aaronson, S. A., Todaro, G. J. & Scolnick, E. M. (1971) *Science* **174,** 157–159.
9. Lowy, D. R., Rowe, W. P., Teich, N. & Hartley, J. W. (1971) *Science* **174,** 155–156.
10. Gelb, L. D., Milstien, J. B., Martin, M. A. & Aaronson, S. A. (1973) *Nature New Biol.* **244,** 76–79.
11. Chattopadhyay, S. K., Lowy, D. R., Teich, N. M., Levine, A. S. & Rowe, W. P. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 167–171.
12. Benveniste, R. E. & Todaro, G. J. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4513–4518.
13. Benveniste, R. E., Sherr, C. J. & Todaro, G. J. (1975) *Science* **190,** 886–888.
14. Tronick, S. R., Golub, M. M., Stephenson, J. R. & Aaronson, S. A. (1977) *J. Virol* **23,** 1–9.
15. Huebner, R. J., Kelloff, G. J., Sarma, P. S., Lane, W. T. & Turner, H. C. (1970) *Proc. Natl. Acad. Sci. USA* **67,** 366–376.
16. Parks, W. P., Livingston, D. M., Todaro, G. J., Benveniste, R. E. & Scolnick, E. M. (1973) *J. Exp. Med.* **137,** 622–635.
17. Stephenson, J. R., Tronick, S. R., Reynolds, R. K. & Aaronson, S. A. (1974) *J. Exp. Med.* **139,** 427–438.
18. Stephenson, J. R. & Aaronson, S. A. (1977) *Nature* **260,** 469–472.
19. Benveniste, R. E., Lieber, M. M., Livingston, D. M., Sherr, C. J., Todaro, G. J. & Kalter, S. S. (1974) *Nature* **248,** 17–20.
20. Goldberg, R. J., Scolnick, E. M., Parks, W. P., Yakovleva, L. A. & Lapin, B. A. (1974) *Int. J. Cancer* **4,** 722–730.
21. Chopra, H. C. & Mason, M. M. (1970) *Cancer Res.* **30,** 2081–2068.
22. Jensen, E. M., Zelljadt, I., Chopra, H. C. & Mason, M. M. (1970) *Cancer Res.* **30,** 2388–2393.
23. Benveniste, R. E. & Todaro, G. J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4557–4561.
24. Drohan, W., Colcher, D., Schochetman, G. & Schlom, J. (1977) *J. Virol.* **23,** 36–43.
25. Heberling, R. L., Barker, S. T., Kalter, S. S., Smith, G. C. & Helmke, R. J. (1977) *Science* **195,** 289–292.
26. Theilen, G. H., Gould, D., Fowler, M. & Dungworth, B. L. (1971) *J. Natl. Cancer Inst.* **47,** 881–889.
27. Kawakami, T. G., Huff, S. D., Buckley, P. M., Dungworth, D. L., Synder, S. P. & Gilden, R. V. (1972) *Nature New Biol.* **235,** 170–171.
28. Lasfargus, E. Y., Kramarsky, B. & Sarkar, N. M. (1972) *Proc. Soc. Exp. Biol. Med.* **139,** 242–247.
29. Parks, W. P., Scolnick, E. M., Todaro, G. J. & Aaronson, S. A. (1971) *Nature* **229,** 258–260.
30. Schincariol, A. L. & Joklik, W. W. (1973) *Virology* **56,** 532–548.
31. Britten, R. J., Graham, D. E. & Neufeld, B. R. (1974) in *Methods in Enzymology,* eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 29, pp. 363–418.
32. Benveniste, R. E. & Scolnick, E. M. (1973) *Virology* **51,** 370–382.
33. Leong, J. A., Garapin, A. C., Jackson, H., Fanshier, L., Levinson, W. & Bishop, J. M. (1972) *J. Virol.* **9,** 891–903.
34. Hino, S., Stephenson, J. R. & Aaronson, S. A. (1976) *J. Virol.* **18,** 933–941.
35. Fleissner, E. (1971) *J. Virol.* **8,** 778–785.
36. Greenwood, F. C., Hunter, W. M. & Glover, J. S. (1963) *Biochem. J.* **89,** 114–123.
37. Colcher, D., Heberling, R. L., Kalter, S. S. & Schlom, J. (1977) *J. Virol.* **23,** 294–301.
38. Gilden, R. V., Oroszlan, S. & Huebner, R. J. (1971) *Nature New Biol.* **231,** 107–198.
39. Barbacid, M., Stephenson, J. R. & Aaronson, S. A. (1977) *Cell* **10,** 641–648.
40. Krakower, J. M., Barbacid, M. & Aaronson, S. A. (1977) *J. Virol.* **22,** 331–339.
41. Barbacid, M., Stephenson, J. R. & Aaronson, S. A. (1976) *J. Biol. Chem.* **251,** 4859–4866.
42. Tronick, S. R., Stephenson, J. R. & Aaronson, S. A. (1974) *J. Virol.* **14,** 125–132.
43. Devare, S. G., Stephenson, J. R., Sarma, P. S., Aaronson, S. A. & Chander, S. (1976) *Science* **194,** 1428–1430.
44. Parks, W. P., Howk, R. S., Scolnick, E. M., Oroszlan, S. & Gilden, R. V. (1974) *J. Virol.* **13,** 1200–1210.
45. Romer, A. S. (1966) *Vertebrate Palentology* (Univ. Chicago Press, Chicago and London), 3rd Ed.
46. Colcher, D., Teramoto, Y. A. & Schlom, J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5739–5743.