WILD MOUSE RNA TUMOR VIRUSES

A Nongenetically Transmitted Virus Group Closely Related to Exogenous Leukemia Viruses of Laboratory Mouse Strains*

By MARIANO BARBACID, KEITH C. ROBBINS,‡ AND STUART A. AARONSON

From the Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20014

Investigations aimed at elucidating the mechanisms involved in leukemogenesis associated with type-C RNA viruses have focused increasingly upon the mouse model. This is to a large extent due to the availability of inbred mouse strains that can be genetically characterized with respect to susceptibility to virus and disease. Type-C virus isolates of laboratory mice can be categorized into two major groups, whose complete genomes can be demonstrated within mouse cellular DNA (endogenous viruses) and whose complete genomes cannot (exogenous viruses). The relatively few examples of the latter were isolated years ago from animals after serial passage of tumors (1–3). These viruses have each been shown to be highly oncogenic, causing lymphomas in susceptible hosts.

Recently, investigations have been undertaken to study the role of type-C viruses in naturally occurring diseases of outbred wild mouse (*Mus musculus*) populations. Gardner and coworkers have described the isolation of type-C viruses that are causative of naturally occurring spontaneous lymphoma and even paralytic neurologic diseases in certain demes of wild mice (for review, see reference 4). The potential importance of the wild mouse as a model for type-C virus-associated malignancies has led us to characterize the prototype wild mouse viruses with respect to their genetic relationship to the mouse cell and to known exogenous and endogenous viruses of laboratory mouse strains.

Materials and Methods

Cells and Viruses. Cells were grown in Dulbecco's modification of Eagle's medium supplemented with 10% calf serum (Colorado Serum Co., Denver, Colo.). The continuous mouse NIH/3T3 (5) and dog sarcoma D17 (R. M. McAllister, University of Southern California) cell lines were utilized. Wild mouse (WM) 292 and 1504 type-C viruses isolated by Gardner and coworkers (6, 7) were provided by R. J. Huebner, National Cancer Institute. The WM amphotropic (292-A and 1504-A) and ecotropic (292-N) components (8–10) were isolated by repeated end-point dilution in D17 and NIH/3T3 cells, respectively. Other viruses utilized included Friend-(1), Moloney-(2), and Rauscher-(3) murine leukemia virus (MuLV), BALB:virus-1 (11), BALB:virus-2 (11), and NIH-MuLV (12).

Antisera. Antisera against type-C viruses isolated from laboratory strains of mice were

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‡ Present address: Hazelton Laboratory, Vienna, Va. 22180.

prepared by immunizing goats with Tween (Atlas Chemical Industries, Inc., Wilmington, Del.)-ether disrupted preparations of isopycnically banded viruses. Swine antiserum against amphotropic WM 292-A type-C virus was obtained by immunization with autologous fibroblasts infected in vitro with WM 292-A virus. These antisera were provided by R. Wilsnack (Huntingdon Research Center, Baltimore, Md.) through the Virus Cancer Program, National Cancer Institute.

Preparation of Viral ^3H cDNA. Viral cDNA transcripts were prepared from sucrose gradient-purified virus in reaction mixtures containing 0.02 M Tris-HCl, pH 7.8; 0.02 M KCl; 0.001 M dithiothreitol; 0.006 M $MgCl_2$; 10^{-4} M deoxyadenosine 5′-triphosphate, deoxycytidine 5′-triphosphate, and deoxyguanosine 5′-triphosphate; 10^{-5} M ^{3}H-thymidine 5′-triphosphate (50 Ci/mmol); 0.014% Triton X-100; 0.02 mg/ml actinomycin D; 0.1 mg/ml virus, and 1 mg/ml DNase I-digested calf thymus DNA (13). Reactions were terminated after 6 h incubation at 37°C by addition of 0.2 M Tris-HCl, pH 7.5; 0.2 M NaCl; 0.05 M EDTA; 0.8% sodium dodecyl sulfate; and 1 mg/ml self-digested pronase. ^{3}H-labeled viral cDNA was purified as previously described (14). Less than 2% of the purified ^{3}H-cDNA was resistant to degradation by S1 nuclease. The specific activity of viral cDNA probes was 2×10^7 cpm/mg.

The genetic complexity of viral cDNA was estimated by its ability to protect ^{32}P-labeled homologous 70S RNA from RNase digestion (15). At a DNA:RNA ratio of 1:1, homologous RNA was hybridized 40 and 50%, respectively, by WM 292-N and 292-A viral cDNAs. At a DNA:RNA ratio of 5:1, the homologous RNA was hybridized 50 and 65%, respectively.

Molecular Hybridization. Viral 70S RNA was prepared as previously described (16). Cellular DNA was purified by a modified Marmur procedure as described by Britten et al. (17) and was sheared to an average size of 6–8S in a Virtis model 60K homogenizer (Virtis Inc., Gardner, N. Y.). The preparation of ^{3}H-labeled unique sequence cellular DNA has also been reported (18). RNA-DNA hybridization was performed in 0.5 ml reaction mixtures containing ≅20,000 cpm of viral cDNA, 0.01 M Tris-HCl, pH 7.5; 0.6 M NaCl; 0.001 M EDTA; and saturating amounts of viral RNA. For DNA-DNA hybridization, 0.5 ml reaction mixtures contained 2 mg of DNA; 12,000 cpm of ^{3}H-cDNA; 0.01 M Tris-HCl, pH 7.5; 0.6 M NaCl; and 0.001 M EDTA. After heat dissociation, hybridization was performed at 62°C. At varying times, 0.025-ml aliquots were quick-frozen and stored at −70°C. Hybridization was assayed by the S1 nuclease method (19, 20). The thermal stability of hybrids formed between cDNA and cellular DNA or viral RNA was determined by heating aliquots of the reaction mixture at 5° increments from 65–95°C for 3 min followed by digestion with S1 nuclease. C_0t and C_rt are, respectively, the product of the initial concentration of DNA or RNA in moles of nucleotide per liter and incubation time in seconds.

Purification of WM 292-A Type-C Viral Structural Proteins. *gag*-gene coded p15, p12, and p30 proteins of the WM 292-A type-C virus were purified by ion-exchange phosphocellulose (Whatman P11, Whatman, Inc., Clifton, N. J.) chromatography as described elsewhere (21). Elution of *gag*-gene coded proteins was monitored by testing appropriate fractions in group-specific radioimmunoassays able to specifically detect the antigenic determinants of each protein. WM 292-A p12, which eluted in the wash of the phosphocellulose column, was purified to homogeneity by gel filtration chromatography in the presence of 6 M guanidine-HCl (22, 23). Fractions containing partially purified WM 292-A p15 (those eluting between 0.3–0.5 M KCl) were pooled, concentrated to 1 ml by ultrafiltration, and applied to an Ultragel Aca 34 (LKB, Instruments Inc., Bromma, Sweden) column (1.5 × 90 cm) (21). Pure WM 292-A p15 (as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE]) was found to elute with an apparent mol wt of 85,000 as previously observed with other mouse type-C viral p15 proteins (24). Homogeneous WM 292-A p30, as determined by electrophoretic criteria, was found to elute from the phosphocellulose column in fractions containing 0.6–0.8 M KCl. Finally, the *env* gene-coded gp70 glycoprotein was purified by a combination of affinity and anion-exchange chromatography as described (25). All purified viral proteins were aliquoted and stored under liquid nitrogen.

Radioimmunoassays. Purified viral proteins were labeled to high specific activity (30–80 μCi/μg) with ^{125}Iodine by the chloramine T method of Greenwood et al. (26). They included the *gag* gene-coded p15, p12 and p30 proteins of WM 292-A virus, Rauscher-MuLV, Moloney-MuLV, BALB:virus-1; BALB:virus-2, and NIH-MuLV as well as the *env* gene-coded gp70 of WM 292-A type-C virus.

Immunoprecipitation assays were performed essentially as previously described (27). Twofold serial dilutions of the appropriate antiserum were incubated for 3 h at 37°C with 10,000 cpm of ^{125}I-labeled viral protein in a 0.2-ml reaction mixture containing 0.01 M Tris-HCl, pH 7.8; 0.001 M EDTA; 0.4% Triton X-100; 0.6% bovine serum albumin (fraction V; Sigma Chemical Co., St. Louis, Mo.); 0.001 ml of undiluted nonimmunized serum and either 0.01 M NaCl (p12 and p30 immunoassays) or 0.3 M NaCl (p15 and gp70 immunoassays). After an overnight incubation in the cold, 0.025 ml of undiluted antiserum, prepared against immunoglobulins of the species of origin of the tested antiserum, were added and incubated for another 3 h in the cold. Samples, diluted to 1 ml with the above buffer without bovine serum albumin, were centrifugated (2,500 rpm for 15 min). Supernates were aspirated, and the radioactivity in the precipitate was measured in a Searle 1285 gamma counter (Searle Diagnostics Inc., subsid. of G. D. Searle & Co., Des Plaines, Ill.). Double-antibody competition radioimmunoassays were performed similarly except that twofold serial dilutions of the tested sample were preincubated for 1 h at 37°C with a dilution of the corresponding antiserum able to precipitate 25% of the input ^{125}I-labeled viral protein (27).

Detection of Type-C Viral p12 Antigenic Reactivity in Wild Mouse Tissues. Partial purification of type-C viral p12 proteins from wild mouse livers was performed by methods previously described (28) with minor modifications. 15 livers, obtained from 3-mo-old wild mice (*M. musculus*) maintained as a colony at Microbiological Associates (Bethesda, Md.) were disrupted in 1 vol of 0.01 M Tris-HCl buffer, pH 7.8, 0.001 M EDTA, and 0.1% Triton X-100 with a blender. The concentration of Triton X-100 was then raised to 0.5% and the tissues homogenized in a Potter-Elvehjem homogenizer (Potter Instrument Co., Inc., Plainview, N. Y.) with Teflon piston. The resulting extract was clarified by centrifugation (2,500 rpm for 30 min), precipitated with 50% (wt/vol) ammonium sulphate, and resuspended in 1/6 vol of 0.01 M Tris-HCl buffer, pH 8.5, 0.001 M EDTA, and 0.1% Triton X-100 (TET buffer). After dialysis to remove ammonium sulphate, soluble proteins were applied to a DEAE-cellulose (Whatman DE 52, Whatman, Inc., Clifton, N. J.) column (2.5 × 90 cm) equilibrated with TET buffer and eluted with 1 liter of a linear KCl gradient (0–0.5 M KCl) in TET buffer. Fractions containing p12 antigenic reactivity, as determined in the anti-BALB:virus-2: ^{125}I-labeled Rauscher-MuLV p12 group-specific assay, were pooled, lyophilized, and resuspended in 2 ml of 0.05 M Tris-HCl buffer, pH 8.0, containing 8 M guanidine-HCl, and 0.02 M dithiothreitol. After a 15-min incubation at 37°C, soluble proteins were applied to an A1.5-m agarose (Bio-Rad Laboratories, Richmond, Calif.) column (1.5 × 90 cm). Fractions were extensively dialyzed against a 0.01 M Tris-HCl buffer, pH 7.8, containing 0.05 M NaCl, 0.001 M EDTA, and 0.1% Triton X-100 and tested as competing antigens in appropriate immunoassays as described in the text. Protein determinations were performed according to Lowry et al. (29), using bovine serum albumin as standard.

Results

Infectious Type-C Viruses Isolated from Wild Mice Are Not Endogenous. To study the representation of wild mouse amphotropic virus nucleotide sequences within mouse cellular DNA, the ability of WM 292-A virus-infected NIH/3T3 cellular DNA to hybridize a cDNA transcript of the prototype WM 292-A amphotropic virus was first examined. As shown in Fig. 1, DNA from virus-infected cells annealed 72% of the probe at a C_0t of 5×10^3, whereas uninfected NIH/3T3 cell DNA hybridized only 35% of the same cDNA. These findings suggested that uninfected NIH/3T3 cells lacked nucleotide sequences represented within the WM 292-A virus genome.

From the kinetic analysis of results in Fig. 1, canine D17 cells infected with the WM 292-A virus demonstrated around one to two proviral copies, whereas WM 292-A virus-infected NIH/3T3 cells contained around three copies per haploid genome. The nucleotide sequences partially related to the WM 292-A viral cDNA probe in uninfected NIH/3T3 cell DNA were present in multiple copies suggesting that these represented sequences of endogenous viruses present within the cells. When WM 292-A virus-infected NIH/3T3 cell DNA was diluted 10-times with DNA of uninfected

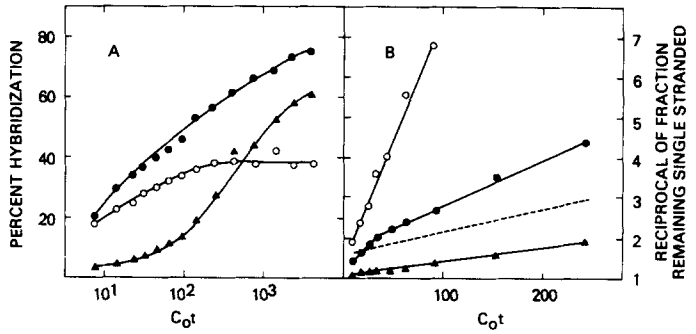

FIG. 1. Detection of amphotropic wild mouse type-C viral sequences in cellular DNAs. Association kinetics of ^{3}H-labeled WM 292-A viral cDNA with DNAs from NIH/3T3 (○), WM 292-A infected NIH/3T3 (●), and WM 292-A infected D17 (▲) cells were plotted as (A) percent hybridization and as (B) the reciprocal of the fraction of cDNA remaining single stranded (48). The kinetics of self-association of unique sequence mouse cellular DNA (dashed line) are included as a standard defining one copy per haploid genome.

TABLE I

Hybridization of WM 292-A Type-C Viral ^{3}H-cDNA by Mouse Cellular DNA

DNA source	Maximum* hybridization	T_m(°C)‡	ΔT_m(°C)§
Cell lines			
WM 292-A virus-infected NIH/3T3	72	90.6	0
NIH/3T3	35	82.7	7.9
Wild mouse embryo	36	83.2	7.4
Tissues			
Wild mouse	36	81.0	9.6
Inbred strains			
NIH	35	82.5	8.1
C58	42	81.2	9.4
C57L	36	83.3	7.3
NZB	37	81.1	9.5
SWR	36	80.5	10.1

* Results are expressed as the final extent of hybridization at a C_0t of 10^4 and represent mean values from three separate determinations.

‡ Thermal stability of hybrids formed between WM 292-A viral cDNA and cellular DNA was determined as described in Materials and Methods.

§ ΔT_m is the difference from the T_m of the hybrids formed between viral cDNA and DNA from WM 292-A infected NIH/3T3 cells.

NIH/3T3 cells, approximating 0.3 proviral copy per haploid genome, the maximum hybridization was still 58%. This value was significantly higher than that obtained by hybridization with uninfected NIH/3T3 DNA alone. These findings established that the sensitivity of the hybridization conditions was sufficient to detect less than a single copy of integrated wild mouse amphotropic virus DNA per haploid genome had it been present in NIH/3T3 cells.

The T_m of hybrids formed between WM 292-A virus-infected NIH/3T3 cellular DNA and the WM 292-A ^{3}H-cDNA probe was 90.6°C, indicating the formation of well-matched hybrids. In contrast, the T_m of hybrids formed with DNA of uninfected NIH/3T3 cells was appreciably lower (Table I). These results excluded the possibility that the WM 292-A virus-related nucleotide sequences detected in NIH/3T3 cells

represented a conserved segment of the WM 292-A viral genome.

We next investigated whether the wild mouse amphotropic virus was represented within the cellular genomes of wild mice as well as within cellular DNAs of several inbred mouse strains. As shown in Table I, saturating amounts of wild mouse liver cell DNA annealed only as much as 35% of the WM 292-A viral cDNA probe. Similar findings were obtained with DNAs of representative inbred mouse strains. Maximum hybridization values ranged from 35 to 40% and were independent of the number and types of endogenous viruses known to be present in each strain. In each case, the low T_ms of the hybrids substantiated the conclusion that the nucleotide sequence homology observed was due to partially related but not identical viral information present within genomes of the mouse strains analyzed. Analogous studies performed with the ecotropic WM 292-N isolate also indicated that this virus was not naturally integrated within the genomes of either wild or inbred strains of mice (data not shown). All of these findings demonstrate that the amphotropic and ecotropic type-C virus isolates of wild mice are not endogenous to *M. musculus*.

Subviral Expression in Normal Wild Mouse Tissues. The presence of multiple copies of mouse type-C virus-related nucleotide sequences in DNA of wild *M. musculus* prompted us to investigate the expression of viral gene products in normal tissues of uninfected wild mice. Wild mouse liver tissues were homogenized and fractionated by sequential ammonium sulphate precipitation and ion-exchange chromatography as described in Materials and Methods. Fractions exhibiting group-specific antigenic reactivity in a mouse viral p12 immunoassay were submitted to gel-filtration chromatography in the presence of 6 M guanidine-HCl. Immunoreactive protein(s) eluted with an apparent mol wt of 12,000 demonstrating that type-C viral p12 was expressed normally in wild mouse tissues.

To determine whether this p12 protein was coded for by the wild mouse type-C viral genomes, we developed an homologous competition immunoassay for the p12 structural protein of the WM 292-A virus, in which the amphotropic and ecotropic wild mouse viruses competed to full extents. (Fig. 2). In contrast, the partially purified p12 protein from wild mouse tissues exhibited almost no antigenic cross-reactivity. Similar results were obtained in Rauscher- and Moloney-MuLV p12 homologous assays (data not shown). These findings indicate that the viral p12 protein detected in normal wild mouse tissues does not correspond to that of exogenous viruses but rather is coded for by as yet unidentified endogenous proviral sequences of the wild mouse.

Amphotropic and Ecotropic Wild Mouse Type-C Viruses Are env Gene Variants. We next investigated the genetic relationships between different type-C viruses isolated from wild mice. Viruses were grouped according to two criteria, viruses possessing similar host range but isolated from animals trapped in separate geographical areas (WM 292-A and 1504-A) and viruses isolated from the same mouse but exhibiting differential host range (WM 292-A and 292-N) (8–10). The extent of nucleotide sequence homology between these viruses was determined by hybridizing their 70S RNAs to ^{3}H-cDNA transcripts of the WM 292-A and 292-N genomes. As shown in Table II, both amphotropic WM 292-A and 1504-A viruses, exhibited extensive if not total, homology. In contrast, only 70–80% hybridization was observed when the genome of the WM 292-N virus was compared with those of either WM 292-A or 1504-A amphotropic isolates (Table II). The high thermal stability of the hybrids formed between the genomes of the ecotropic and amphotropic wild mouse viruses suggested

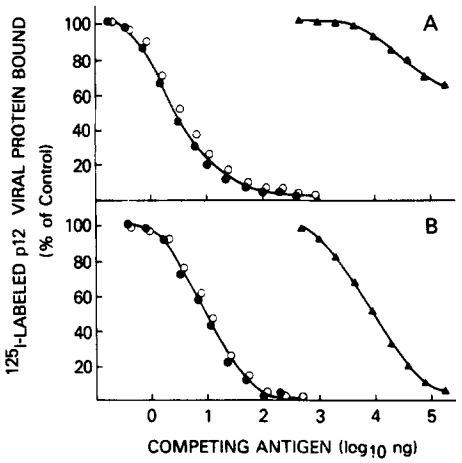

FIG. 2. Immunoreactivity of partially purified type-C viral p12 protein from wild mouse tissues. Soluble proteins from homogenized wild mouse livers were submitted to ammonium sulphate precipitation and subsequently fractionated by DEAE-cellulose and gel-filtration chromatography in the presence of 6 M guanidine-HCl (see Materials and Methods). Fractions eluting with an apparent mol wt of 12,000 from the gel-filtration column (▲) as well as sucrose-gradient purified WM 292-A (●), and WM 292-N type-C viruses (○) were tested at twofold serial dilutions as competing antigens in the (A) type-specific WM 292-A p12 and in the (B) group-specific anti BALB:virus-2: ^{125}I-labeled Rauscher-MuLV p12 radioimmunoassays. The results are expressed as the percent of total ^{125}Iodine counts per minute in the antigen-antibody precipitate normalized to 100% for maximal binding at infinite competing antigen dilution. Protein determinations were performed by the method of Lowry et al. (29).

TABLE II

Nucleic Acid Homologies Between WM 292 Viral ^{3}H-cDNAs and the Genomes of Several Mouse Type-C Viruses

70S RNA source	WM 292-A ^{3}H-cDNA		WM 292-N ^{3}H-cDNA	
	Maximum* percent hybridization	ΔT_m‡	Maximum* percent hybridization	ΔT_m‡
WM 292-A type-C virus	96	0	71	0.2
WM 292-N type-C virus	81	0.3	98	0
WM 1504-A type-C virus	98	0.3	72	0.1
Rauscher-MuLV	69	6.0	81	7.3
Moloney-MuLV	71	6.9	72	7.1
BALB: virus-1	45	8.6	59	6.4
BALB: virus-2	50	10.1	36	8.2
NIH-MuLV	41	9.5	38	6.1

* Results are expressed as the final extent of hybridization at a C_rt of 0.5 and represent mean values from three separate determinations.

‡ ΔT_m is the difference in T_m between homologous and heterologous viral cDNA-70S RNA hybrids.

that their genetic differences might be localized to a specific region of their viral RNAs.

To further study the genetic relationships between these viruses, homologous competition radioimmunoassays were developed for other *gag* gene products, including p15 and p30, as well as for the *env* gene-coded gp70 glycoprotein of the amphotropic

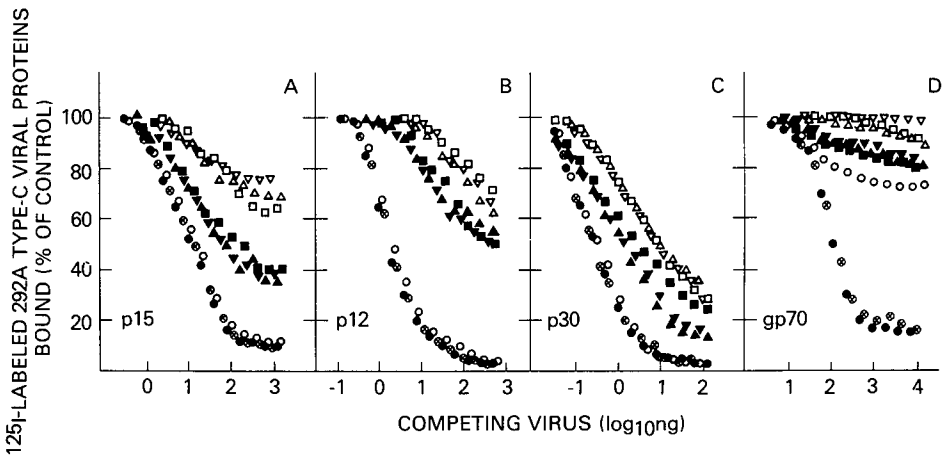

FIG. 3. Analysis of immunologic relatedness of type-C viruses isolated from wild and laboratory mice. Sucrose gradient-purified mouse type-C viruses, including WM 292-A (●), WM 292-N (○), WM 1504-A (⊗), Friend-MuLV (▲), Moloney-MuLV (■), Rauscher-MuLV (▼), BALB:virus-1 (△), BALB:virus-2 (□), and NIH-MuLV (▽), were tested at twofold serial dilutions as competing antigens in homologous radioimmunoassays for the (A) p15, (B) p12, (C) p30, and (D) gp70 proteins of WM 292-A type-C virus.

WM 292-A type-C virus. As shown in Fig. 3, each molecule exhibited antigenic determinants that readily distinguished it from analogous proteins of type-C viruses previously isolated from laboratory mice. Wild mouse type-C viruses, including the amphotropic 1504-A and ecotropic 292-N isolates, appeared to possess *gag* gene-coded proteins immunologically indistinguishable from those of the WM 292-A virus.

Analysis of their envelope glycoproteins indicated that WM 1504-A and WM 292-A gp70s were highly related. In contrast, the ecotropic WM 292-N virus exhibited only a limited number of related antigenic determinants. These findings, along with those obtained by molecular hybridization, support the concept that wild mouse amphotropic viruses isolated from neighboring trapping areas were very similar, if not identical. However, the ecotropic and amphotropic viruses isolated from the same wild mouse represent very closely related viruses that have diverged, at least, in the *env* gene regions of their respective genomes.

Relationships between Wild Mouse Viruses and Those Isolated from Laboratory Strains of Mice. The exogenous nature of the wild mouse type-C viruses raised the question of their relationship to viruses isolated from laboratory mouse strains. Molecular hybridization studies revealed that the 70S RNAs of Rauscher- and Moloney-MuLVs hybridized a large extent (70%) of the ^{3}H-cDNA probe complementary to the WM 292-A viral genome (Table II). Even higher values were obtained when the same RNAs were used to hybridize a ^{3}H-cDNA transcript of WM 292-N genome. In the same series of experiments, only 40–60% of either probe was annealed by 70S RNA purified from ecotropic or xenotropic endogenous mouse type-C viruses (Table II).

The greater homology between wild mouse isolates and non-endogenous as compared to endogenous type-C viruses isolated from laboratory mice was further demonstrated by radioimmunologic techniques. As shown in Fig. 3, viruses of the FMR subgroup (30–32) shared 60% of the WM 292-A p15 antigenic determinants detected in the homologous immunoassay for this protein. These viruses also shared

TABLE III

Immunologic Relationships Between the gag Gene-Coded p12 Proteins of Mouse Type-C Viruses

Competition radioimmunoassay	Percent Displacement of ^{125}I-labeled WM 292-A p12 Protein By:*							
	Exogenous viruses					Endogenous viruses		
	Wild mouse		FMR subgroup			Ecotropic	Xenotropic	
	292-A	292-N	M-MuLV	R-MuLV	F-MuLV	AKR-MuLV	BALB-v-2	NIH/MuLV
Anti-WM 292-A:								
^{125}I WM 292-A p12	100	100	50	48	45	35	25	30
Anti-M-MuLV:								
^{125}I WM 292-A p12	100	100	100	40	37	25	25	40
Anti-R-MuLV:								
^{125}I WM 292-A p12	100	100	100	100	100	30	25	60
Anti-AKR-MuLV:								
^{125}I WM 292-A p12	100	100	100	100	100	100	100	95
Anti-BALB-v-2:								
^{125}I WM 292-A p12	100	100	100	100	100	100	100	100
Anti-NIH-MuLV:								
^{125}I WM 292-A p12	100	100	100	100	100	93	95	100

* Isopycnically banded type-C viruses were tested at twofold serial dilutions for their ability to displace ^{125}I-labeled WM 292-A p12 viral protein (10,000 cpm/tube) for binding limiting amounts of antiserum raised against the corresponding mouse type-C virus. Results indicate percent competition achieved by 10 μg of a given virus with respect to that observed with WM 292-A virus (95–100%).

around 50% of the highly type-specific determinants of the WM 292-A viral p12 protein, and >80% of those present in the well-conserved p30 major internal protein of WM 292-A virus. In contrast, the extent of antigenic homology of the corresponding p15, p12 and p30 *gag* gene-coded proteins of several prototype endogenous mouse type-C viruses was only 30, 35, and 60%, respectively (Fig. 3). In reciprocal studies, there was significantly greater antigenic homology of WM 292 viruses in type-specific immunoassays for the *gag* gene-coded proteins of Moloney- and Rauscher-MuLVs (≧75, 70, and 90% homology between their p15, p12, and p30 proteins, respectively), than in the corresponding type-specific immunoassays for BALB:virus-1, BALB:virus-2, and NIH-MuLV prototype endogenous viruses (≧40, 30, and 70% homology between their p15, p12, and p30 proteins, respectively).

The same experimental approach was utilized to establish the immunologic relatedness of WM 292-A virus gp70 with the envelope glycoproteins of other mouse type-C viruses. All tested viruses, including exogenous and endogenous isolates, exhibited only very limited competition in the homologous WM 292-A viral gp70 immunoassay (Fig. 3 D). Similarly, both WM 292 viruses competed poorly (<50% displacement of the ^{125}I-labeled gp70 probe) in homologous radioimmunoassays for the envelope glycoproteins of prototype exogenous and endogenous viruses of laboratory mice (data not shown). These findings indicate a higher degree of immunologic divergence among the *env* gene-products of wild and laboratory mouse type-C viruses than between their corresponding *gag* gene-coded proteins.

Evolutionary Pathways of Endogenous and Exogenous Mouse Type-C Viruses. The above studies suggested a close relationship between exogenous viruses of wild and laboratory mice. However, these findings were not sufficient to define the precise evolutionary relationships of these viruses to those endogenous to the mouse cell. Such conclusions would require demonstration of the existence of genetic markers shared by only a subset of viruses.

For this purpose, we designed several competition immunoassays in which the

highly type-specific ^{125}I-labeled WM 292-A viral p12 protein was precipitated with limiting amounts of antisera raised against representative type-C viruses isolated from laboratory mice. As shown in Table III, the p12 antigenic determinants common to wild mouse viruses and each of the prototype endogenous viruses, were present in all tested *M. musculus* type-C viruses. Thus, these well-conserved determinants must represent those derived from a putative common progenitor. In contrast, most of the determinants shared by the WM 292 virus isolates and the Rauscher and Moloney strains of exogenous MuLVs were not present in the prototype endogenous viruses (Table III). These findings indicate that the exogenous viruses isolated from wild and laboratory mice are evolutionarily more closely related to each other than to those naturally integrated within the mouse cell genome. Furthermore, the lack of complete competition of both Rauscher- and Friend-MuLV in the anti-Moloney MuLV: ^{125}I-WM 292-A p12 assay established Moloney-MuLV as the laboratory strain, of all tested murine leukemia viruses, most closely related to the prototype wild mouse viruses utilized in the present studies.

Discussion

Type-C viruses isolated from mice trapped in the wild have been shown to be causative of spontaneous lymphoma and paralytic neurological diseases (33–35). These findings established that naturally occurring viruses of unselected, outbred wild mouse populations possess oncogenic potential. Epidemiologic studies by Gardner and coworkers have indicated that congenital infection is a major route of virus transmission. Infectious virus can be readily isolated from the maternal genital tract, and there is a highly significant maternal contribution to the early expression of viremia (4). These studies, however, have not indicated whether the wild mouse viruses are genetically transmitted.

The ability of normal cellular DNA to hybridize a radioactive viral cDNA prepared by reverse transcription of type-C viral RNA is considered as evidence for the presence of endogenous virus-related information in such cells (36, 37). To establish that a specific virus is completely represented as an endogenous virus, in the absence of evidence of its inducibility, rigorous criteria must be fulfilled. The cDNA probe should be shown to represent a significant portion of the viral genome. Moreover, the extent of hybridization, as well as the thermal stability of the hybrids formed with cellular DNA of the species in question, must be the same as that defined by experiments utilizing DNAs from experimentally virus-infected cells. Nucleotide sequences of related, but not identical, endogenous viruses exhibit lower extents of hybridization and greater base-pair mismatching as determined by lower melting temperatures of the hybrids formed.

In the present report, it was possible to demonstrate that wild and laboratory mouse cellular DNAs contain nucleotide sequences partially related, but not identical to either prototype wild mouse amphotropic or ecotropic viral genomes. These findings are at variance with those of a recent report, indicating that the wild mouse amphotropic virus is endogenous to both wild and laboratory mouse strains (38). However, conclusions in this latter study were based upon the use of a cDNA probe that was not characterized with respect to its representation of the viral genome. Moreover, homologous and heterologous virus-infected cell DNAs were not used as controls. That prototype wild mouse viruses are not endogenous to *M. musculus* implies

both that their persistence in the animal is dependent solely upon nongenetic transmission, and that these viruses cannot be considered as progenitors of the present endogenous viruses of laboratory mouse strains.

Amphotropic and ecotropic wild mouse viruses isolated from the same mouse possessed indistinguishable antigenic determinants in their *gag* gene-coded proteins, but differed markedly in the immunologic properties of their *env* gene-coded glycoproteins. These results are in agreement with previous studies in which the tryptic peptides of the p12 and gp70 proteins of wild mouse viruses were analyzed (39, 40). Molecular hybridization studies indicated the formation of well-matched hybrids between the two viral genomes despite nucleotide sequence homology of only ≅70%. These findings argue that the wild mouse viruses differ in a specific region of their viral genomes that can be localized to the *env* gene. This might reflect a more rapid evolutionary divergence of this region of the viral genome as a consequence of host cell adaptation of replicating viruses. Alternatively, either the ecotropic or amphotropic virus might have arisen as a result of a recombinational event involving the other virus and an, as yet, unidentified endogenous type-C virus present within the wild mouse cellular genome.

A group of early type-C virus isolates, Friend-, Rauscher-, and Moloney-MuLV, form a subgroup of closely related laboratory strains (31, 32, 41–43). These viruses, like the wild mouse viruses studied here, lack complete representation of their genomes within mouse cellular DNA (44, 45). Thus, the origin of FMR subgroup viruses has remained unresolved. In the present studies, it was possible to demonstrate a high degree of genetic homology between FRM subgroup viruses and prototype wild mouse viruses by molecular hybridization and radioimmunologic techniques. More importantly, these viruses were shown to share a subset of antigenic determinants not present in any of the prototype endogenous type-C viruses of mouse origin. These findings, taken together, indicate a closer evolutionary relationship of wild mouse and FMR subgroup exogenous viruses to each other than to known endogenous viruses of mouse cells.

The present day exogenous viruses of wild and laboratory mice may have arisen from endogenous viruses that recently escaped host cell regulatory controls that once restricted their expression. This hypothesis would require that such viruses have undergone more rapid genomic change than that affecting viruses naturally transmitted within the mouse cell. Evidence against this hypothesis comes from findings that Gross leukemia virus (46), passaged in the laboratory for more than two decades, remains indistinguishable, by available biochemical and immunologic techniques, from the prototype *N*-tropic endogenous mouse virus. Similarly, there has been no detectable genetic alteration in Rauscher-MuLV continuously passaged in vitro for over 8 yr (unpublished observations). Thus, it is unlikely that the exogenous viruses have emerged from within the mouse cell genome within a time period during which laboratory mouse strains have been derived. Instead, as postulated by the evolutionary-clock theory (47), it is more likely that the wild mouse and Friend-, Moloney-, and Rauscher-MuLV subgroup viruses have evolved at a relatively constant rate. The degree of divergence of the exogenous from endogenous viruses would then imply that the nongenetically transmitted viruses have been maintained in nature over a long period of evolution as a separate virus group. Thus, the mouse provides a model in which type-C virus-induced tumors occur by two mechanisms, one involving

derepression of naturally integrated viral genetic information and the other by the more classical transmission of viruses as infectious agents.

Summary

Type-C RNA viruses isolated from wild mice are causative of naturally occurring neoplasia and neurologic diseases. Biochemical and immunologic characterization of this virus group revealed that amphotropic viruses isolated from wild mice trapped in separate geographical areas are indistinguishable, whereas amphotropic and ecotropic viruses naturally infecting the same animal are *env* gene variants. Molecular hybridization studies established that neither host range variant is endogenous to the *Mus musculus* genome, although each demonstrates partial nucleotide sequence homology. Wild mouse type-C viruses exhibited much closer molecular and antigenic relatedness to the exogenous virus subgroup (Friend-, Moloney-, and Rauscher-MuLV) than to prototype endogenous viruses isolated from laboratory mouse strains. The evidence indicates that exogenous mouse type-C viruses have been maintained in nature over a long period of evolution as a separate virus group, causative of tumors in mice by a mechanism solely involving their transmission as infectious agents.

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