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Acquisition of Oncogenicity by Endogenous Mouse Type C Viruses: Effects of Variations in *env* and *gag* Genes

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Several dual-tropic isolates derived from the thymuses of preleukemic or leukemic AKR mice and a more recent group of viruses generated by in vitro or in vivo passage of a poorly infectious endogenous virus of C3H mouse cells have been shown to be highly oncogenic. By analysis of the immunological properties of their *gag* gene-coded structural proteins, each of the AKR-derived isolates and two dual-tropic C3H-derived isolates were found to closely resemble AKR murine leukemia virus. In contrast, *gag* gene-coded proteins of two other leukemogenic isolates of C3H origin, including one ecotropic and one dual-tropic virus, were indistinguishable from those of Moloney murine leukemia virus. All of the oncogenic isolates, including those of AKR and C3H origin, were found to possess common envelope glycoprotein determinants of a unique class not shared by the nononcogenic ecotropic viruses from which they were derived. These findings support the possibility that oncogenic variants of endogenous ecotropic mouse type C viruses are derived by genetic recombination. This recombinational event appears to involve the acquisition, by different ecotropic viruses, of a common class of endogenous virus-coded envelope glycoprotein determinants which are presumably required, but not necessarily sufficient, for oncogenicity.

Evidence for the etiological involvement of mammalian type C viruses in tumors of their natural hosts has been derived to a large extent from studies of endogenous type C viruses of mice (1, 12). Although numerous type C virus isolates of murine origin have been described, only certain of these are leukemogenic in vivo (3, 14). Recently, a unique class of viruses, obtained from preleukemic and leukemic thymuses of AKR mice, was shown to produce characteristic cytopathic foci in cultured mink lung cells. Such viruses have been designated as MCF (mink cell focus-inducing) strains (8, 10). They are, in general, leukemogenic when inoculated into newborn mice of the AKR strain and exhibit dual host ranges—they are ecotropic as well as xenotropic (8). A second class of rapid-leukemia-inducing viruses has been derived by both in vivo and in vitro passage of a poorly infectious ecotropic virus released by the C3H/10T1/2 mouse embryo fibroblast cell line after iododeoxyuridine activation (14). This latter group of viruses includes isolates which produce large XC plaques on mouse cells but do not grow on mink cells, as well as isolates which resemble viruses of the MCF group in that they replicate well in both mouse and mink cells and produce morphological alteration of mink cells. Biochemical characterization of the envelope glycoproteins of several AKR-derived MCF isolates by tryptic peptide analysis has indicated that viruses of this class may have arisen as a result of genetic recombination within their envelope glycoprotein (*env*) genes (5). The present studies were undertaken to compare these different groups of highly leukemogenic viruses. For this purpose, competition immunoassays known to discriminate analogous structural proteins of prototype murine type C isolates were used.

MATERIALS AND METHODS

Viruses. The virus strains used in the present study are described in Table 1. With the exception of NIH murine leukemia virus (NIH-MuLV), which was propagated in the human rhabdomyosarcoma line A673 (17), and AKR-MuLV, which was spontaneously released by cultured AKR embryo fibroblasts (9), all viruses were grown in the wild mouse cell line SC-1. Viruses were isolated from tissue culture fluids by sucrose density gradient centrifugation.

PAGE. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) was performed by the method of Laemmli (11). Acrylamide gels were either stained with Coomassie brilliant blue or divided into 1-mm fractions, and radioactivity was measured in a Searle model 1285 gamma counter.

Isolation of type C virus *env* (gp70)- and *gag*

TABLE 1. *Description of type C virus isolates*

Virus isolate	Description	Leukemogenicity[a]	
		Inoculation in newborn NIH Swiss mice	AKR leukemia acceleration test
Ecotropic			
AKR-MuLV	XC^+ virus spontaneously released by a continuous AKR embryo fibroblast culture (9)	SL	SL
M-MuLV	XC^+ virus propagated on NIH/3T3 after in vitro and in vivo passage in BALB/c mouse cells (13)	SL/RL	NT
A-2	XC^- virus spontaneously released by iododeoxyuridine-treated C3H/10T1/2 cells (14)	NL	NT
NIH clone 6	XC^+ virus isolated from thymus tissue after passage of A-2 in newborn Swiss mice (14)	RL	NT
Xenotropic			
NIH-MuLV	Endogenous virus isolated by in vivo passage of a human tumor cell line, A673, in immunosuppressed NIH Swiss mice (17)	NL	NL
Dual-tropic			
AKR-247	XC^- virus isolated from thymus of 6-month-old AKR mouse (8)	NT	RL
AKR-MB36	XC^- virus isolated from leukemia tissue of an NIH Swiss mouse partially congenic for Akv-1 (Hartley and Rowe, unpublished data)	NT	SL
AKR-13	XC^- virus isolated from thymus of 3-month-old AKR mouse that had received a thymus graft from 6-month-old AKR mouse (5)	NT	RL
Z-6	XC^- virus isolated from thymus tissue after passage of A-2 in newborn NIH Swiss mice (14)	RL	NT
Z-9	XC^- virus isolated by end point cloning in mink cells from leukemic thymus of an NIH Swiss mouse that had been inoculated at birth with an XC^+ slowly leukemogenic virus derived by in vitro passage of A-2 in methylcholanthrene-transformed C3H/10T1/2 cells (14)	SL	NT
FC clone 3	XC^- focus-derived virus isolated from transformed mink cells that had been inoculated with leukemic tissue after passage of an XC^+ slowly leukemogenic virus derived by in vitro passage of A-2 in methylcholanthrene-transformed C3H/10T1/2 cells (14)	SL	NT

[a] Leukemogenicity was tested either by inoculation into newborn NIH Swiss mice (14) or, in the cases of AKR-247, AKR-MB36, and AKR-13, by indirect injection in thymuses of AKR mice (leukemia acceleration test; J. W. Hartley and W. P. Rowe, unpublished data). NL, Nonleukemogenic (no leukemias after 18 months); SL, slowly leukemogenic (leukemias developing later than 9 months after inoculation); RL, rapidly leukemogenic (leukemias developing in less than 4 months). One virus isolate, M-MuLV, induced leukemias at an intermediate average latent period (5 to 7 months).

(p15, p12)-coded structural proteins. Approximately 25 mg of density gradient-purified virus was disrupted by sonic treatment for 20 s in 0.05 M Tris-hydrochloride (pH 8.5) buffer containing 0.5% Triton X-100, clarified by centrifugation at 100,000 × *g* for 30 min, and subjected to agarose-lentil lectin (P-L Biochemicals, Inc., Milwaukee, Wis.) column chromatography. For this purpose, the virus lysate was applied to an agarose-lentil lectin column (1.5 by 5.0 cm) and washed with 0.01 M sodium phosphate (pH 7.4)–0.15 M NaCl buffer at 4°C until free of absorbance at 280 nm, and the bound glycoprotein was eluted with 0.1 M α-methyl-mannopyranoside at room temperature. Fractions containing the 70,000- to 80,000-molecular-weight viral envelope glycoprotein (gp70), as determined by SDS-PAGE (11), were pooled, dialyzed against TET buffer (10 mM Tris [pH 7.8], 0.2 mM EDTA, 0.5% Triton X-100, and applied to a DEAE-cellulose (Whatman; H. Reeve Angel and Co., Clifton, N.J.) column (1.5 by 5.0 cm) equilibrated with the same buffer. The column was washed with TET buffer, and bound protein was eluted with a linear 0.0 to 1.0 M NaCl gradient. Fractions containing gp70 were pooled, aliquoted, and stored under liquid nitrogen.

After dialysis against 200 volumes of BET buffer (0.01 M *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid [pH 6.5], 0.5 mM EDTA, 0.1% Triton X-100), proteins in the wash fractions of the lectin column were applied to a phosphocellulose column (1.5 by 5.0 cm; Whatman P11; Reeve Angel) previously equilibrated with the same buffer (19). The column was washed with 50 ml of BET buffer, and bound proteins were eluted with 100 ml of a 0.0 to 1.0 M NaCl linear gradient. Fractions containing p15 (0.4 to

0.5 M NaCl), as determined by SDS-PAGE analysis, were lyophilized, suspended in 0.5 ml of 0.025 M Tris-hydrochloride (pH 7.8)–0.5 mM EDTA–0.1 M NaCl buffer containing 0.1% Triton X-100, and subjected to further purification by acrylamide-agarose gel filtration column chromatography (18). Proteins eluting in the wash of the phosphocellulose column, which included p12, were lyophilized, suspended in 0.5 ml of 0.05 M Tris-hydrochloride (pH 8.5)–0.01 M dithiothreitol–2 mM EDTA buffer containing 8 M guanidine hydrochloride, and applied to an agarose A-5m (100 to 200 mesh) gel filtration column (1.5 by 90 cm; Bio-Rad Laboratories, Richmond, Calif.) in the presence of 6 M guanidine hydrochloride (7). Column fractions eluting at a molecular weight of 12,000 relative to standards were exhaustively dialyzed against 0.01 M Tris-hydrochloride (pH 7.8)–0.2 mM EDTA–0.05 M NaCl–0.1% Triton X-100 buffer, aliquoted, and stored under liquid nitrogen.

Competition immunoassays. Purified viral proteins were labeled with ^{125}I at high specific activity (5 to 20 $\mu Ci/\mu g$) by the previously described iodogen procedure (4). Competition immunoassays were performed by testing unlabeled antigens at serial twofold dilutions for ability to compete with ^{125}I-labeled viral proteins for binding limiting amounts of goat antiserum prepared against detergent-disrupted virus. Reaction mixtures contained 0.01 M Tris-hydrochloride (pH 7.8), 1.0 mM EDTA, 0.4% Triton X-100, 1% bovine serum albumin, and 0.05 M NaCl in a total volume of 0.2 ml. In assays in which either gp70 or p15 was used as the ^{125}I-labeled antigen, the NaCl concentration was increased to 0.3 M. Antiserum and unlabeled competing antigen were incubated at 37°C for 1 h, followed by addition of 10,000 cpm of ^{125}I-labeled antigen. After incubation for 3 h at 37°C and 18 h at 4°C, 0.025 ml of undiluted pig anti-goat immunoglobulin G was added to each tube to precipitate antigen-antibody complexes, and samples were incubated further for 1 h at 37°C and 3 h at 4°C. After the addition of 0.4 ml of cold 10 mM Tris-hydrochloride (pH 7.8)–10 mM NaCl buffer containing 0.5% Triton X-100, the samples were centrifuged for 15 min at 2,500 rpm, the supernatants were aspirated, and the radioactivity in the precipitates was quantitated in a Searle model 1285 gamma counter.

RESULTS

Type-specific competition immunoassay for MCF envelope glycoprotein determinants. The major 70,000-molecular-weight envelope glycoproteins (gp70's) of AKR-MuLV, NIH-MuLV, and the prototype MCF isolate, AKR-247(MCF), were purified and labeled at high specific activity with ^{125}I as described above. The radiochemical purity of labeled proteins is shown in Fig. 1 by their migration by SDS-PAGE analysis as single homogeneous bands at molecular weights of about 70,000. Preservation of the immunological reactivity of viral envelope glycoproteins after purification was indicated by the fact that all were immunoprecipitable at high titer and to final extents of over 90% by their respective homologous goat antiserum (data not shown).

In an initial effort to characterize the AKR-derived MCF viruses, isolates were tested in homologous AKR-MuLV, NIH-MuLV, and AKR-247(MCF) gp70 competition immunoassays (Fig. 2). AKR-MuLV and NIH-MuLV were included in each assay as controls. In contrast to AKR-MuLV, which competed efficiently and to a final extent of over 95% in the AKR-MuLV gp70 assay, each of the three AKR-derived MCF isolates competed only partially (<50%). In a second assay utilizing antiserum to NIH-MuLV for precipitation of ^{125}I-labeled NIH-MuLV gp70, NIH-MuLV competed efficiently, whereas neither AKR-MuLV nor any of the MCF isolates exhibited more than a minor extent of cross-reactivity. Finally, in the homologous MCF gp70 competition assay, each of the MCF isolates competed efficiently and to a final extent of close to 100%, whereas AKR-MuLV was considerably less reactive (<60% maximum competition), and NIH-MuLV displaced only 20% of the ^{125}I-labeled antigen even at the highest concentration tested. These findings demonstrate that each of the AKR-derived MCF viruses possesses shared envelope glycoprotein antigenic determinants of a class which are distinct from those exhibited by either AKR-MuLV or NIH-MuLV.

Analysis of *gag* gene-coded structural proteins of AKR-derived MCF viruses. In view of the above findings, it was of interest to immunologically analyze the *gag* gene-coded structural components of virus isolates of the MCF group. For this purpose the highly type-specific, 12,000-molecular-weight structural proteins (p12's) of AKR- and NIH-MuLV were purified and ^{125}I labeled at high specific activity as described above. Both labeled proteins migrated as single homogeneous peaks by SDS-PAGE (Fig. 3) and were over 90% immunoprecipitable by homologous high-titered antisera (data not shown). As shown in Fig. 4, detergent-disrupted AKR- and NIH-MuLV competed efficiently and to high titer in their respective homologous immunoassays. In contrast, AKR-MuLV was only weakly reactive in the NIH-MuLV p12 assay, and, conversely, NIH-MuLV competed to only a limited extent in the AKR-MuLV p12 assay. Each of the MCF isolates was found to compete equally as efficiently as AKR-MuLV in the homologous AKR-MuLV p12 assay. Similarly, in a homologous competition immunoassay for AKR-247(MCF) p12, AKR-MuLV and each of the AKR-derived MCF isolates competed efficiently and to final extents of over 95% (Table 2), whereas other type C viruses tested, including NIH-MuLV, Rauscher-MuLV, BALB:virus-2, and NZB-MuLV, were only

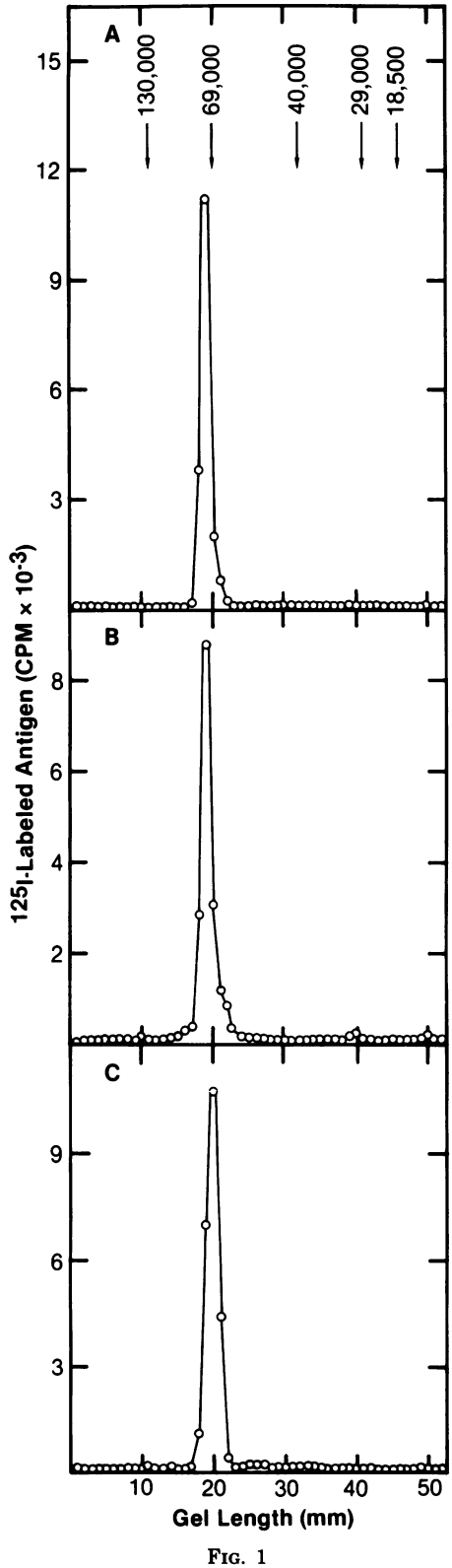

A
B
C
130,000
69,000
40,000
29,000
18,500
^{125}I-Labeled Antigen (CPM x 10^{-3})
Gel Length (mm)

FIG. 1

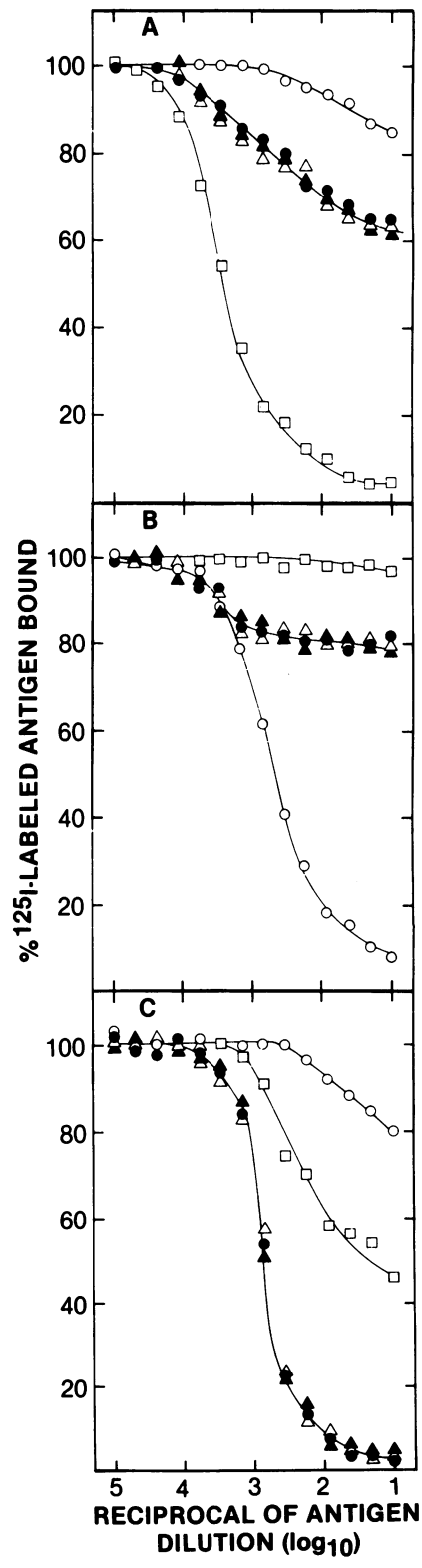

A
B
C
% ^{125}I-LABELED ANTIGEN BOUND
RECIPROCAL OF ANTIGEN DILUTION (log_{10})

FIG. 2

weakly reactive (<50% competition; data not shown).

For further characterization of the *gag* gene-coded components of the above virus isolates, a homologous competition immunoassay for AKR-MuLV p15 was developed. Although this assay was somewhat less type specific than the corresponding AKR-MuLV p12 assay, Fig. 5 clearly indicates that the p15's of the MCF viruses closely resemble AKR-MuLV p15. These results provide strong support for the possibility that the MCF viral p12 and p15's are highly related, or identical, to the corresponding AKR-MuLV structural proteins. Moreover, because p15 and p12 are known to map at the 5′ terminus of the type C viral *gag* gene (2, 15) and evidence has been presented indicating that the MCF viruses are recombinants (5, 16), these findings favor the possibility that the 5′ regions of the MCF viral genomes are AKR-MuLV derived.

Characterization of envelope glycoproteins of leukemogenic type C viruses derived from a poorly infectious endogenous C3H-derived virus isolate. In addition to providing information regarding the nature of the AKR-derived MCF viruses, the above-described immunological assays provide a means for characterizing the highly leukemogenic C3H-derived viruses. In initial studies, each of these latter viruses, a focus-derived FC clone 3, and A-2, the endogenous C3H isolate from which they were derived, were tested in homologous AKR-MuLV, NIH-MuLV, and AKR-247(MCF) gp70 competition immunoassays. Figure 6 shows that the A-2 isolate exhibited a pattern of reactivity in each of these assays indistinguishable from those of AKR-MuLV. Both AKR-MuLV and A-2 competed efficiently in the AKR-MuLV gp70 assay, but were only weakly reactive in the corresponding AKR-247(MCF) and NIH-MuLV gp70 assays. In contrast, the highly leukemogenic viruses derived by in vivo or in vitro passage of A-2 and the sarcomagenic isolate, FC clone 3, closely resembled the AKR-derived

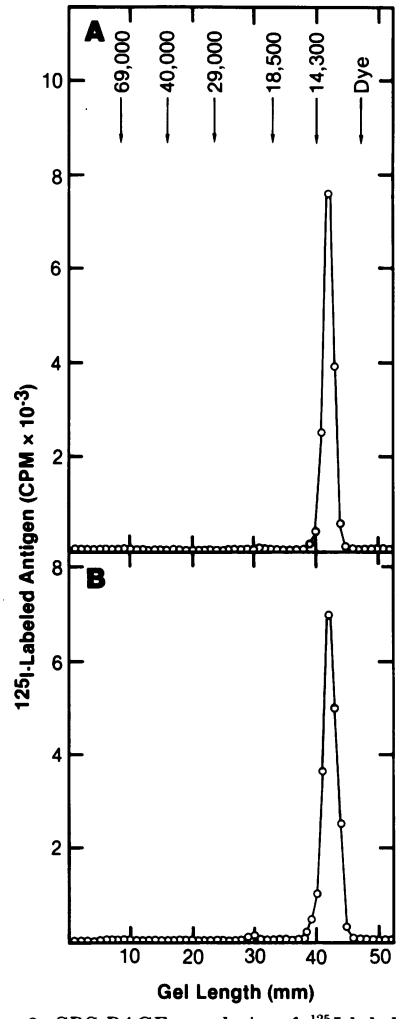


FIG. 3. *SDS-PAGE analysis of ^{125}I-labeled p12 polypeptides. Approximately 25,000 cpm of ^{125}I-labeled (A) AKR-MuLV p12 and (B) NIH-MuLV p12 were subjected to electrophoresis on 55-mm SDS-polyacrylamide gels (12%) at 2.5 mA/gel for 3 h. Molecular weight standards and analysis of gels were as described in the legend to Fig. 1.*

FIG. 1. *SDS-PAGE analysis of ^{125}I-labeled envelope glycoproteins. Approximately 25 to 30,000 cpm of ^{125}I-labeled (A) AKR-MuLV gp70, (B) NIH-MuLV gp70, and (C) AKR-247(MCF) gp70 were subjected to electrophoresis on 55-mm SDS-polyacrylamide gels (7.5%) at 2.5 mA/gel for 2 h. After electrophoresis, samples were either stained with Coomassie brilliant blue or sliced into 1-mm fractions and tested for radioactivity in a Searle model 1285 gamma counter. Molecular weight standards used for calibration included β-galactosidase (130,000), bovine serum albumin (69,000), alcohol dehydrogenase (40,000), carbonic anhydrase (29,000), and β-lactoglobulin (18,500).*

FIG. 2. *Homologous competition immunoassays for murine type C viral 70,000-molecular-weight envelope glycoproteins. Unlabeled detergent-disrupted viruses were tested at serial twofold dilutions for the ability to compete with (A) ^{125}I-labeled AKR-MuLV gp70 for binding limiting amounts of antiserum to AKR virus, (B) ^{125}I-labeled NIH-MuLV gp70 for binding limiting amounts of antiserum to NIH virus, and (C) ^{125}I-labeled AKR-247(MCF) gp70 for binding limiting amounts of antiserum to AKR-247(MCF). Results are expressed as mean values from two separate determinations and are normalized to 100% for maximal binding at infinite antigen dilution. Viruses tested as competing antigens included: AKR-MuLV (□), NIH-MuLV (○), AKR-247(MCF) (△), AKR-MB36(MCF) (▲), and AKR-13 (MCF) (●).*

MCF isolates. Each of these latter viruses competed to much greater extents (>90%) in the AKR-247(MCF) gp70 assay than in either the AKR-MuLV or NIH-MuLV assay.

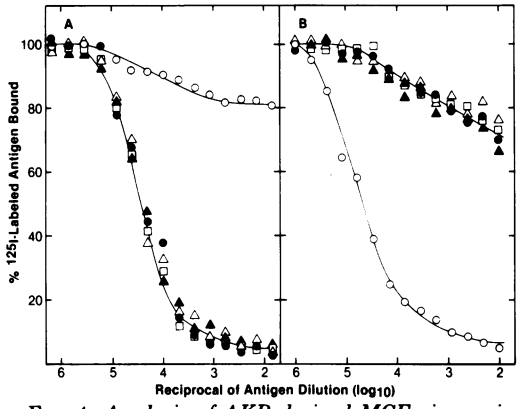


FIG. 4. *Analysis of AKR-derived MCF viruses in homologous p12 competition immunoassays. Unlabeled detergent-disrupted viruses were tested at serial twofold dilutions for the ability to compete with (A) ^{125}I-labeled AKR-MuLV p12 for binding limiting amounts of antiserum to AKR-MuLV and (B) ^{125}I-labeled NIH-MuLV p12 for binding limiting amounts of antiserum to NIH-MuLV. Symbols for viruses tested as competing antigens are as described in the legend to Fig. 2.*

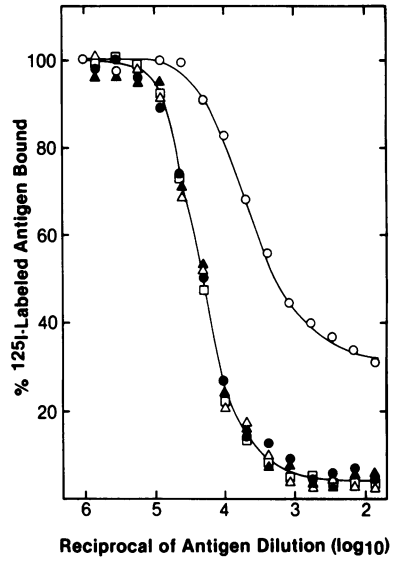
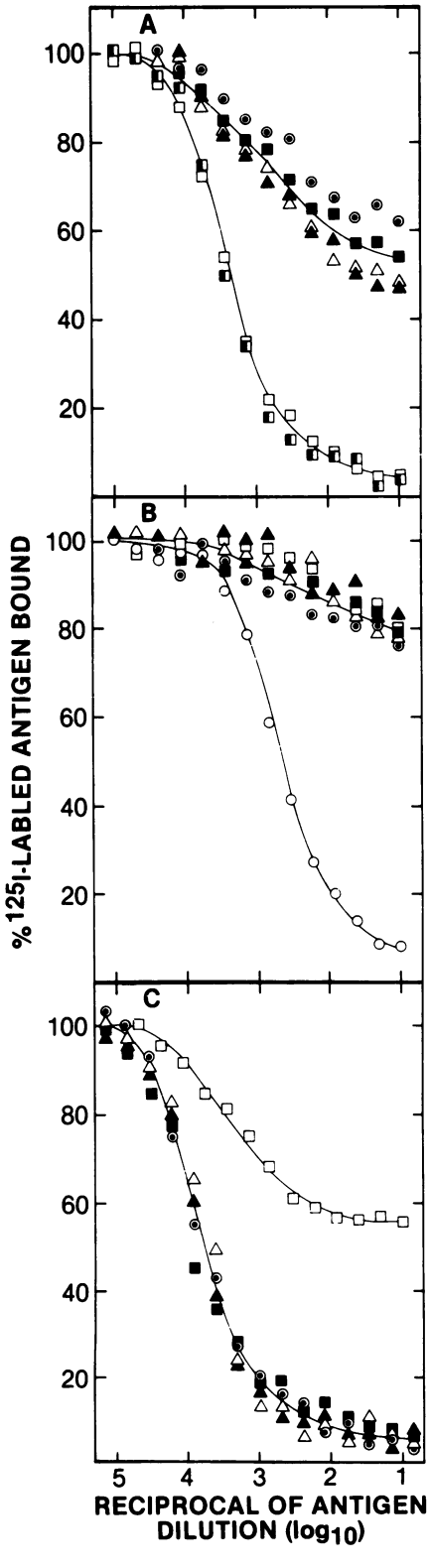


FIG. 5. *Analysis of AKR-derived MCF viruses in a homologous competition immunoassay for AKR-MuLV p15. Unlabeled detergent-disrupted viruses were tested at serial twofold dilutions for the ability to compete with ^{125}I-labeled AKR-MuLV p15 for binding limiting amounts of goat antiserum to AKR-MuLV. Symbols for viruses tested as competing antigens are as described in the legend to Fig. 2.*

TABLE 2. *Immunological characterization of highly leukemogenic type C virus isolates of mouse origin*

Virus isolate	% Competition in homologous immunoassays for[a]:								
	gp70				p15	p12			
	AKR-MuLV	AKR-247 (MCF)	NIH-MuLV	M-MuLV	AKR-MuLV	AKR-MuLV	AKR-247 (MCF)	NIH-MuLV	M-MuLV
Ecotropic									
AKR-MuLV	[95]	54	3	35	[96]	[95]	[96]	27	14
M-MuLV	42	33	21	[97]	48	12	10	13	[98]
A-2	[95]	44	19	26	[94]	[92]	[93]	19	5
NIH clone 6	38	[97]	24	80	45	34	32	41	[96]
Xenotropic									
NIH-MuLV	15	20	[92]	24	69	20	18	[95]	11
Dual-tropic									
AKR-247(MCF)	36	[98]	21	34	[94]	[96]	[94]	23	19
AKR-MB36(MCF)	37	[97]	22	32	[95]	[92]	[93]	26	12
AKR-13(MCF)	36	[98]	20	33	[94]	[95]	[92]	24	14
Z-6	46	[94]	21	52	37	33	23	60	[97]
Z-9	51	[95]	22	29	[92]	[95]	[92]	17	10
FC clone 3	53	[92]	17	30	[94]	[92]	[95]	29	14

[a] Detergent-disrupted viruses were tested in homologous competition immunoassays as described in the text. Results are expressed as the level of competition achieved at the highest virus concentration (1 mg/ml) tested. Values of over 90% competition are enclosed in boxes.



Each of the viruses described in Table 1 were also tested in a homologous competition immunoassay for Moloney MuLV (M-MuLV) gp70. Interestingly, two C3H-derived viruses obtained by in vivo passage in newborn NIH Swiss mice exhibited unexpectedly high levels of reactivity. These included an ecotropic virus, NIH clone 6, which displaced up to 80% of the labeled antigen at low dilution, and a dual-tropic isolate, Z-6, which competed to a final extent of 52%. These results indicate that oncogenic murine type C viruses of diverse origin share similar envelope glycoprotein determinants. In addition, they raise the possibility that the recombinational event leading to formation of two of these viruses may have involved acquisition of M-MuLV-related genetic sequences.

C3H-derived virus isolates possess *gag* gene components (p12's) in common with either AKR- or M-MuLV. For further characterization of the oncogenic virus isolates of the C3H-derived group, the *gag* gene components of these viruses were immunologically analyzed. For this purpose, each isolate was tested in the AKR-MuLV, AKR-247(MCF), and NIH-MuLV p12 immunoassays, as well as in a highly type-specific assay for M-MuLV p12. As shown in Fig. 7, one leukemogenic virus, Z-9, and the sarcomagenic isolate, FC clone 3, exhibited patterns of reactivity indistinguishable from those obtained with AKR-MuLV. Both viruses competed to essentially 100% in the AKR-MuLV and AKR-247(MCF) p12 assays, but were only weakly reactive in the analogous p12 assays for NIH-MuLV and M-MuLV. In contrast, the two C3H-derived isolates which were partially cross-reactive in the M-MuLV gp70 assay (NIH clone 6 and Z-6) exhibited markedly different patterns of reactivity. Both competed equally as efficiently as M-MuLV in the M-MuLV p12 assay, but were only weakly reactive in the AKR-MuLV, AKR-247(MCF), and NIH-MuLV p12 assays. These findings (summarized in Table 2) demonstrate that although the envelope glycoproteins and the type-specific *gag* gene-coded proteins of several of the C3H-derived viruses

FIG. 6. *Immunological characterization of envelope glycoproteins of virus isolates derived from the endogenous C3H mouse virus A-2. Immunoassays were performed as described in the legend to Fig. 2 and included homologous assays for (A) AKR gp70, (B) NIH-MuLV gp70, and (C) AKR-247(MCF) gp70. Unlabeled detergent-disrupted viruses tested as competing antigens included A-2 (□), NIH clone 6 (◉), Z-6 (■), Z-9 (△), and FC clone 3 (▲). In addition, AKR-MuLV (◧) and NIH-MuLV (○), respectively, were included as positive controls in the homologous AKR-MuLV gp70 and NIH-MuLV gp70 assays.*

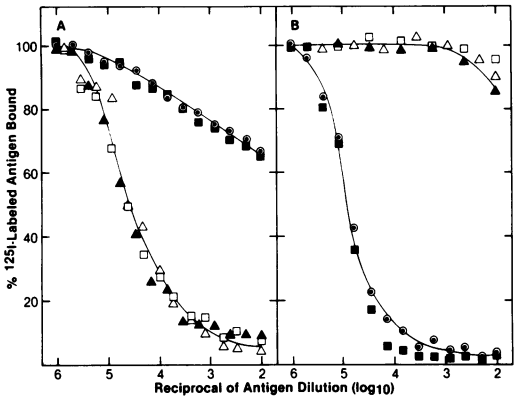


FIG. 7. *Immunological characterization of p12 structural proteins of virus isolates derived from the endogenous C3H mouse virus A-2. Unlabeled detergent-disrupted viruses were tested at serial twofold dilutions for the ability to compete with (A) ^{125}I-labeled AKR-MuLV p12 for binding limiting amounts of antiserum to AKR-MuLV and (B) ^{125}I-labeled M-MuLV p12 for binding limiting amounts of antiserum to M-MuLV. Symbols for viruses tested are as described in the legend to Fig. 6.*

are indistinguishable from those of the AKR-derived MCF viruses, two viruses of the C3H-derived group have sequences, including the *gag* gene and a portion of the *env* gene, analogous to those of M-MuLV.

DISCUSSION

There is increasing evidence that the generation of highly oncogenic type C viruses in mice involves genetic recombination between ecotropic viruses and endogenous viral sequences coding for envelope glycoprotein determinants (5). Such recombinant viruses are generally dual-tropic and frequently cause morphological alteration of mink fibroblasts in cell culture (8, 14). The present findings demonstrate that certain viruses of this nature, including each of three AKR-derived isolates, as well as several isolates derived from a chemically induced virus of C3H origin by prolonged in vitro or in vivo passage, possess *gag* gene-coded proteins immunologically indistinguishable from those of AKR-MuLV. This observation confirms and extends the results of a previous study in which the major structural proteins of each of several AKR-derived MCF isolates were shown to be indistinguishable from those of AKR-MuLV (5). In contrast, other highly leukemogenic viruses, both of C3H origin, including an ecotropic virus, NIH clone 6, and an isolate with a dual-tropic host range, Z-6, exhibited *gag* gene sequences analogous to those of M-MuLV. Each of the highly leukemogenic viruses, including those of both AKR and C3H origin, as well as a C3H-derived virus, FC clone 3, was shown to possess common envelope glycoprotein antigenic determinants of a class not exhibited by either AKR-MuLV or M-MuLV gp70. These findings indicate that such viruses represent genetic recombinants between either AKR-MuLV or M-MuLV and *env* gene sequences of a specific endogenous type C virus of mouse cells.

The results of the present study in combination with those of Rommelaere et al. (16) provide suggestive evidence that the recombinational events leading to the generation of leukemogenic type C viruses of mouse origin may occur at different sites within the *env* region of the viral genome. This is indicated in the present study by the demonstration that of the two highly leukemogenic M-MuLV-related viruses examined, the ecotropic isolate, NIH clone 6, reacted to a greater extent in the M-MuLV glycoprotein assay than did the dual-tropic isolate, Z-6. By characterization and mapping of RNase T1-resistant oligonucleotides, Rommelaere et al. (16) have shown that individual MCF recombinant virus isolates possess related, but not identical, sequences.

One question raised by the present findings relates to the origin of viral genetic sequences coding for the M-MuLV *gag* protein, p12, exhibited by two of the highly leukemogenic isolates. The poorly infectious XC$^-$ virus, from which these isolates were obtained, exhibited *gag* gene-coded structural proteins, similar to the corresponding AKR-MuLV proteins but readily distinguishable from those of M-MuLV. Thus, either the original virus stock must have contained a minority population of M-MuLV-like virions which were amplified as a result of in vivo passage, or, alternatively, an M-MuLV-like virus may have been acquired during passage. The first possibility appears unlikely since repeated attempts to isolate XC-positive, replication-competent virus from the C3H/10T1/2 phase III culture which produces the XC-negative, replication-deficient, mouse-tropic C3H virus have failed. It should be noted that the dual-tropic M-MuLV-like isolate, Z-6, described in the present study appears in certain respects to resemble a previously reported dual-tropic virus acquired by passage of a clonal isolate of M-MuLV in the Swiss mouse cell line 3T3 FL (6).

The results of the present study may provide insight into factors influencing the leukemogenic potential of endogenous type C viruses of mice. For instance, each of the highly leukemogenic isolates was shown to possess a common class of envelope glycoprotein determinants. The converse, however, was not true in that several isolates with dual host ranges and MCF cross-

reactive glycoprotein determinants were only weakly leukemogenic. These findings suggest that a recombinant glycoprotein may be required, although not necessarily sufficient, for leukemogenicity. The fact that certain murine type C viruses with long-term passage history, such as M-MuLV, exhibit greater leukemogenicity than do endogenous ecotropic mouse type C viruses, such as AKR-MuLV, may reflect greater in vivo infectivity or the presence of low levels of contaminating highly leukemogenic variants. One model which would account for the findings of the present study is that the acquisition of endogenous viral envelope glycoprotein determinants may result in enhanced leukemogenicity only if the recombinational event occurs at a specific location within or near the *env* gene. Alternatively, leukemogenicity by a spontaneous or chemically activated ecotropic virus may involve acquisition of not only specific glycoprotein determinants, but also a transforming or *leuk* gene(s) The glycoprotein determinants may be necessary for factors such as the thymotropism which is reported to be characteristic of type C viruses with in vivo leukemogenic potential (3, 10), whereas the *leuk* gene would be necessary to code for one or more proteins required for malignant transformation of lymphoid cells.

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