

TYPE-SPECIFIC RADIOIMMUNOASSAYS FOR THE gp70s OF  
MINK CELL FOCUS-INDUCING MURINE LEUKEMIA VIRUSES:  
EXPRESSION OF A CROSS-REACTING ANTIGEN IN CELLS  
INFECTED WITH THE FRIEND STRAIN OF THE SPLEEN  
FOCUS-FORMING VIRUS

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Extensive studies have recently been reported on the viruses present in thymic leukemia in AKR mice and on the viruses in the Friend virus complex which induce erythroid leukemia. In the AKR system, mink cell focus-inducing viruses (MCF)<sup>1</sup> were cloned from complex mixtures of viruses in pre-leukemic and leukemic thymic tissue (1, 2). By tryptic peptide analysis, the glycoproteins of these MCF viruses were shown to be intragenic envelope gene recombinants with portions of their gp70s derived from both ecotropic parental virus and a mouse xenotropic virus (3). In the Friend system, the highly leukemogenic spleen focus-forming virus (SFFV) was cloned free of helper-independent mouse type-C virus in rat cells (4, 5), and then studied by molecular hybridization. The genome of SFFV was shown to be a recombinant between Friend murine leukemia virus (MuLV) and xenotropic-related genetic sequences which are homologous to the envelope (*env*) gene acquired in the formation of the MCF-type viruses (5, 6). Although nucleic acid studies demonstrated that SFFV contains *env* gene sequences, immunological assays have not been able to detect in fibroblasts nonproductively infected with SFFV a gene product potentially coded for by these sequences.

In the preceding paper, we reported the isolation of two strains of MCF virus derived from ecotropic Friend MuLV. These Friend MCF viruses, like SFFV, are *env* gene recombinants between Friend MuLV and a mouse xenotropic virus. Since the Friend (Fr) MCF viruses are helper-independent viruses (as opposed to SFFV which is replication-defective), we have been able to isolate the gp70 of one of these ecotropic-xenotropic recombinants, Fr-MCF-1. We report here the utilization of the <sup>125</sup>I-labeled gp70 of the Fr-MCF-1 virus to develop an MCF-specific radioimmunoassay which detects specific immunological determinants of the gp70s of recombinant MCF-type viruses not previously detectable in ecotropic- or xenotropic-specific immunoassays. Using this MCF-specific assay, we can now detect a cross-reacting antigen in rat cells infected with the Friend strain of the SFFV. The results provide proof that SFFV codes for an antigen related to the *env* gene product of a recombinant murine type-C virus, and further strengthens the thesis that there may be a common origin for SFFV- and MCF-MuLV.

<sup>1</sup> *Abbreviations used in this paper:* *eco*, ecotropic; *env*, envelope; F or Fr, Friend; FeLV, feline leukemia virus; FeLV/KiSV, FeLV propagated in a KiSV-transformed mink cell; FRE, Fisher rat embryo; MCF, mink cell focus-inducing; Mol, Moloney; MuLV, murine leukemia virus(es); NRK, normal rat kidney; SFFV, Friend strain of spleen focus-forming virus; SR-V-NRK, cells derived from a rat leukemia virus-producing subclone of NRK cells transformed by Schmidt-Ruppin strain of avian sarcoma virus; TP buffer, 0.01 M sodium phosphate buffer, pH 7.6, and 0.1% Triton X-100.

## Materials and Methods

*Viruses and Cells.* Sources of the ecotropic strains of Friend (F-MuLV<sub>201</sub>), Moloney (Mol-MuLV<sub>eco</sub>) and AKR (AKR-MuLV) murine leukemia viruses are described in the preceding manuscript. These ecotropic viruses were propagated in either NIH 3T3 cells, normal rat kidney cells (NRK), or Fisher rat embryo cells (FRE CL2), as indicated in specific experiments. Xenotropic viruses used in these studies included BALB:virus-2 and ATS-124, xenotropic viruses derived from BALB/c mice or NIH Swiss mice, respectively, and NZB, a xenotropic virus induced by halogenated pyrimidines from an NZB cell line (a gift from Dr. Janet Hartley, National Institute of Allergy and Infectious Diseases). Each xenotropic virus was grown on dog thymus cells, Cf<sub>2</sub>th. MCF strains of MuLV, including Fr-MCF-1 (see preceding paper), Mol-MCF<sub>83</sub> (6), and AKR-MCF 247 (2), were propagated in either mink lung fibroblasts (CCL64) or FRE cells. The origin of each MCF virus has been previously described. A nonmurine virus, FeLV/KiSV, is a feline leukemia virus propagated in a Kirsten murine sarcoma virus-transformed mink cell. All viruses were used as 1,000-fold concentrates of sucrose density gradient-banded viruses.

Cells containing the Friend strain of the SFFV include SFFV-NRK CL1 (5), a rat cell nonproductively infected with SFFV, and SFFV-FRE/F-MuLV, a rat cell producing SFFV and F-MuLV<sub>201</sub> at approximately a 5:1 ratio (see preceding paper).

Finally, F-MuLV/FRE cells are Fisher rat embryo cells infected with F-MuLV<sub>201</sub>, and SR-V-NRK cells are derived from a rat leukemia virus-producing subclone of NRK cells transformed by the Schmidt-Ruppin strain of avian sarcoma virus. All cells were grown in Dulbecco's modification of Eagle's medium containing either 10% calf serum or 10% fetal calf serum. Cells were routinely assayed for mycoplasma and found to be negative.

*Sera.* Antiserum to Fr-MCF virus was obtained by immunizing rabbits with viable normal rabbit corneal cells infected with Fr-MCF-1 virus. Anti-Mol-MCF serum was obtained by injecting rabbits with Triton-disrupted Mol-MCF<sub>83</sub> virus previously grown on mink cells. Antiserum to Mol-MuLV<sub>eco</sub> was obtained by immunizing rabbits with Triton-disrupted Mol-MuLV<sub>eco</sub> previously grown on SC-1 mouse cells. Goat antisera to ecotropic F-MuLV, FeLV, and BALB:virus-2 were kindly provided by Dr. Roger Wilsnack, Huntingdon Research Labs, Brooklandville, Md. The exact pedigree of those viruses used for the immunizations is not known.

*Absorption of Antisera.* For certain studies it was necessary to use sera that had been absorbed in the following manner. 1 ml of serum that had been heat-inactivated at 56°C for 30 min was reacted with  $\approx 2.0$  mg of freshly banded virus for 1 h at 37°C. The virus was then removed by centrifugation at 100,000 *g* for 1 h.

*Viral and Cellular Extracts.* Virus and cell extracts to be used in competition radioimmunoassays were prepared by treating sucrose density gradient-banded viruses or washed cell packs with 1% Triton X-100 in phosphate-buffered saline, pH 7.6, incubating at 37°C for 30 min, and centrifuging at 10,000 rpm for 10 min to remove any unsolubilized cell debris or nuclei. Protein determinations were carried out by the method of Lowry et al. (7) using bovine serum albumin as a standard.

*Purification of Viral gp70s.* The gp70s from the ecotropic and MCF strains of F-MuLV and Mol-MuLV were isolated by phosphocellulose chromatography using a modification of previously described procedures (8, 9). 10 mg of banded virus was disrupted at 37°C for 5 min with 1% Triton X-100 and 0.5 M NaCl in a buffer containing 0.2 M Tris-hydrochloride, pH 9.0, and 1 mM EDTA. The preparation was then centrifuged in a Beckman type-50 rotor (Beckman Instruments, Inc., Cedar Grove, N.J.) at 36,000 rpm for 30 min to remove undisturbed material, and the resulting supernate was dialyzed against 100 vol of buffer A (10 mM BES, pH 6.5, 1 mM EDTA, 0.05% Triton X-100). The dialyzed lysate was applied to a 1.5  $\times$  15-cm column of phosphocellulose P-11 (Whatman Inc., Clifton, N.J.) that had been equilibrated with buffer A, and was eluted with a linear gradient of 0-1.0 M KCl in buffer A. Elution of the viral structural proteins was monitored by sodium dodecyl sulfate polyacrylamide slab gel electrophoresis and by competition immunoassays for gp70 using previously purified F-MuLV gp70 (a gift from Dr. Dani Bolognesi, Duke Medical Center, Durham, N. C.) and antiserum to the FeLV. The fractions containing the gp70 (0.1-0.2 M KCl) were concentrated by lyophilization and applied to a 2.5  $\times$  90-cm column of Sephadex G-200 equilibrated with buffer A containing 0.15 M KCl. Again, fractions containing the 70,000-dalton glycoprotein were pooled, concen-

trated by lyophilization, and finally dissolved in 1 ml of distilled water.

**Radioimmunoassays for gp70 Polypeptides.** Competition radioimmunoassays were performed as previously described (10). Briefly, purified gp70s were iodinated according to the chloramine-T (11) or iodogen (12) methods with  $^{125}\text{I}$  to comparable specific activities of 5–10  $\mu\text{Ci}/\mu\text{g}$  polypeptide. Antiserum was incubated with varying concentrations of unlabeled competing antigens at 37°C for 2 h in a 0.5-ml reaction mixture containing 0.01 M sodium phosphate buffer, pH 7.6, and 0.01% Triton X-100 (TP buffer) with 1 mM EDTA and appropriate carrier serum.  $^{125}\text{I}$ -labeled gp70 (50,000 cpm) was then added and incubation was continued for 1 h at 37°C, followed by 16 h at 4°C. An aliquot of 0.1 ml of the appropriate undiluted antiglobulin was then added to each tube and the assay was incubated for another hour at 37°C and for 1 h at 4°C. The tubes were then centrifuged at 2,500 rpm for 15 min, washed once with TP buffer, and the radioactivity in the precipitates was measured by counting in an LKB gamma counter.

For sera titrations, immunoprecipitation assays were carried out exactly as above except that the step adding unlabeled competing antigen was eliminated.

## Results

**Immune Precipitation of Various gp70s by Specific Antisera.** To develop specific immunoassays which would discriminate among ecotropic, xenotropic, and MCF gp70s, it was necessary to obtain antisera which would detect type-specific determinants on these proteins. Various antisera to be used in competition radioimmunoassays were tested against the gp70s of ecotropic and MCF strains of F- and Mol-MuLV. The results are shown in Fig. 1. In Fig. 1A, an antiserum prepared in goats was found to precipitate the gp70 of F-MuLV<sub>eco</sub> to a much greater degree than it precipitated the gp70 of Fr-MCF virus. At a  $10^{-4}$  dilution of this serum,  $\cong 30\%$  of the  $^{125}\text{I}$ -labeled F-MuLV gp70 could be precipitated; in contrast,  $<3\%$  of the  $^{125}\text{I}$ -labeled Fr-MCF gp70 was precipitated at a similar dilution of serum. Thus, this antiserum was used without absorption in F-MuLV<sub>eco</sub> type-specific assays. In Fig. 1B, serum made against Mol-MuLV<sub>eco</sub> precipitated almost equally the ecotropic and MCF Moloney gp70s. Thus, this serum was absorbed with Mol-MCF virus as detailed in Materials and Methods before use in type-specific Mol-MuLV<sub>eco</sub> competition assays. After absorption, a 1:50 dilution of this serum precipitated 20% of the  $^{125}\text{I}$ -gp70 of Mol-MuLV<sub>eco</sub> and  $<3\%$  of the  $^{125}\text{I}$ -gp70 of the Mol-MCF virus. In Fig. 1C, antiserum to Fr-MCF virus precipitated the  $^{125}\text{I}$ -labeled Fr-MCF gp70 well, but failed to precipitate the gp70 of F-MuLV<sub>eco</sub>. Thus, it could be used in competition assays without absorption. In Fig. 1D, antiserum to Mol-MCF virus precipitated the gp70s of both Mol-MCF and Fr-MCF much better than the gp70s of either ecotropic parent; it was thus used in competition assays without absorption.

**Competition Radioimmunoassays Specific for the gp70s of Ecotropic MuLVs.** Using iodinated F-MuLV<sub>eco</sub> gp70 and the antiserum prepared against F-MuLV<sub>eco</sub>, an immunoassay specific for the F-MuLV<sub>eco</sub> was developed. Various ecotropic, xenotropic, and MCF strains of MuLV were tested for their ability to compete in this assay. As shown in Fig. 2A, F-MuLV<sub>eco</sub> competed efficiently in its homologous assay. Mol-MuLV<sub>eco</sub> competed to a lesser degree, whereas competition with AKR-MuLV<sub>eco</sub> was insignificant. The NZB xenotropic virus also competed poorly in this assay. Interestingly, the Fr-MCF virus, as well as the MCF strains of Mol-MuLV and AKR-MuLV, failed to compete in this F-MuLV<sub>eco</sub> gp70 assay, suggesting that the type-specific determinant(s) on the F-MuLV<sub>eco</sub> gp70 was lost during the recombinational event leading to the Fr-MCF virus.

A similar result was obtained with Mol-MuLV<sub>eco</sub> in an assay using iodinated Mol-

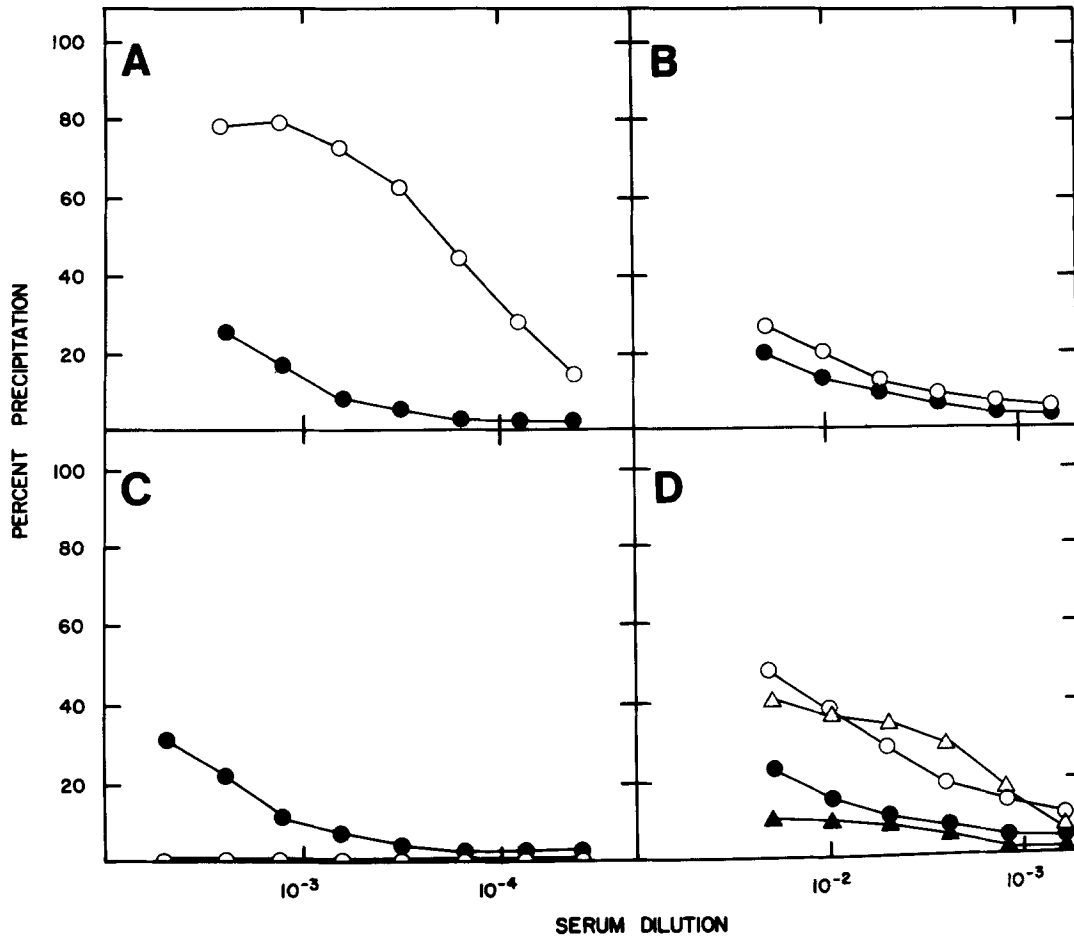


FIG. 1. Immune precipitation of purified gp70s by various antisera.  $^{125}\text{I}$ -labeled gp70s were reacted with various dilutions of anti-virus sera as described in Materials and Methods. (A) Goat antiserum to F-MuLV<sub>eco</sub> was reacted with the gp70 of F-MuLV<sub>eco</sub> (○) or Fr-MCF (●). (B) Rabbit antiserum to Mol-MuLV<sub>eco</sub> was reacted with the gp70 of Mol-MuLV<sub>eco</sub> (○) or Mol-MCF (●). (C) Rabbit antiserum to Fr-MCF virus was reacted with the gp70 of Fr-MCF (●) or F-MuLV<sub>eco</sub> (○). (D) Rabbit antiserum to Mol-MCF was reacted with the gp70 of F-MuLV<sub>eco</sub> (▲), Mol-MuLV<sub>eco</sub> (●), Fr-MCF (△), or Mol-MCF MuLV (○).

MuLV<sub>eco</sub> gp70 and antiserum to Mol-MuLV<sub>eco</sub> which had been absorbed with Mol-MCF virus. As shown in Fig. 2B, Mol-MuLV<sub>eco</sub> competed efficiently in this assay, whereas Friend ecotropic and NZB xenotropic MuLV competed poorly. As in the Friend ecotropic specific gp70 assay, none of the MCF viruses, including the Mol-MCF virus, were able to compete in this assay.

*Comparison of Ecotropic and MCF Viruses in Broadly Reactive Immunoassays.* To assess the group-specific and interspecies-specific determinants of the gp70s of the MCF viruses, both the ecotropic and MCF viruses were tested in assays which would detect the interspecies- or group-specific antigens present on the gp70s. As presented in Fig. 3A, ecotropic and MCF viruses competed with similar efficiency in an interspecies assay utilizing F-MuLV<sub>eco</sub> gp70 and antiserum to the FeLV. The viruses also competed equally well in a group-specific MuLV gp70 assay, Fig. 3B, utilizing Mol-

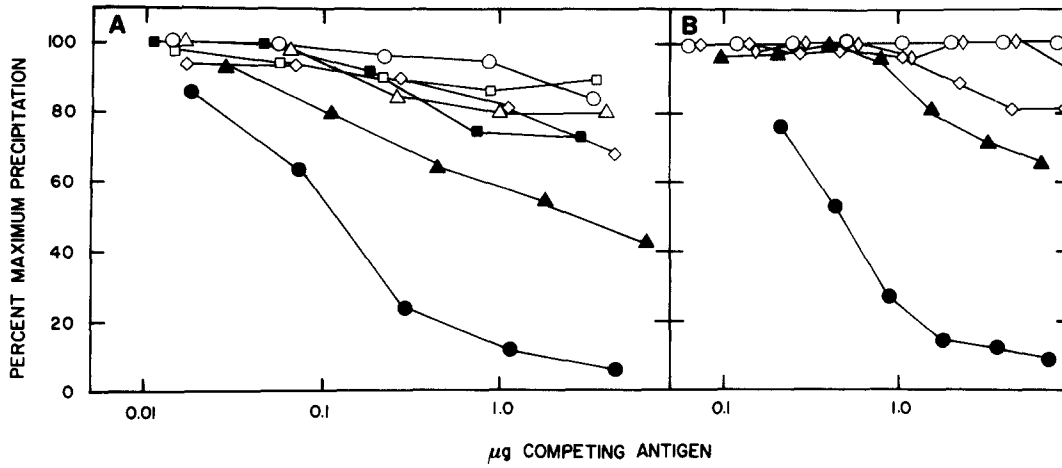


FIG. 2. Reactivities of mouse type-C viruses in type-specific ecotropic assays. Competition radioimmunoassays were carried out as described in Materials and Methods with (A)  $^{125}\text{I}$ -labeled F-MuLV<sub>eco</sub> gp70 and a 1:51,200 dilution of anti-F-MuLV<sub>eco</sub> serum with the following viruses used as competing antigens: F-MuLV<sub>eco</sub> (●); Mol-MuLV<sub>eco</sub> (▲); AKR-MuLV<sub>eco</sub> (■); Fr-MuLV<sub>eco</sub> (○); Mol-MuLV<sub>eco</sub> (△); AKR-MuLV<sub>eco</sub> (□); and NZB xenotropic MuLV (◇). (B)  $^{125}\text{I}$ -labeled Mol-MuLV<sub>eco</sub> gp70 and a 1:50 dilution of anti-Mol-MuLV<sub>eco</sub> serum absorbed with Mol-MuLV<sub>eco</sub> virus, with the following viruses used as competing antigens: Mol-MuLV<sub>eco</sub> (●); F-MuLV<sub>eco</sub> (▲); Mol- or Fr-MuLV<sub>eco</sub> (○); NZB xenotropic MuLV (◇); and ATS xenotropic MuLV (◊).

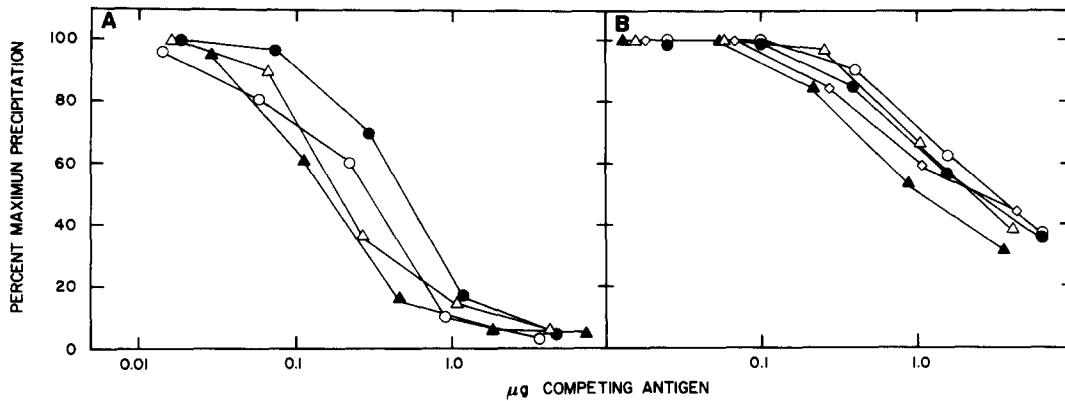


FIG. 3. Comparison of ecotropic and MCF-MuLV in broadly reactive heterologous immunoassays for gp70. Competition radioimmunoassays were carried out as described in Materials and Methods with (A)  $^{125}\text{I}$ -labeled F-MuLV<sub>eco</sub> gp70 and a 1:800 dilution of antiserum to FeLV; or (B)  $^{125}\text{I}$ -labeled Mol-MuLV<sub>eco</sub> gp70 and a 1:50 dilution of antiserum to Mol-MuLV<sub>eco</sub> virus. Competing viruses used in these assays were: Fr-MuLV<sub>eco</sub> (●); Mol-MuLV<sub>eco</sub> (▲); Fr-MuLV<sub>eco</sub> (○); Mol-MuLV<sub>eco</sub> (△); and NZB xenotropic MuLV (◇).

MuLV<sub>eco</sub> gp70 and antiserum to Mol-MuLV<sub>eco</sub> virus. We can therefore conclude that the gp70s of the MCF viruses used in these assays have retained group- and interspecies-specific determinants, although they have lost type-specific determinants of their ecotropic parents.

*Competition Radioimmunoassays Specific for the gp70s of MCF Viruses.* The development of an immunoassay specific for the gp70s of MCF viruses is of obvious general importance and was of particular interest to us in analyzing proteins coded for by the

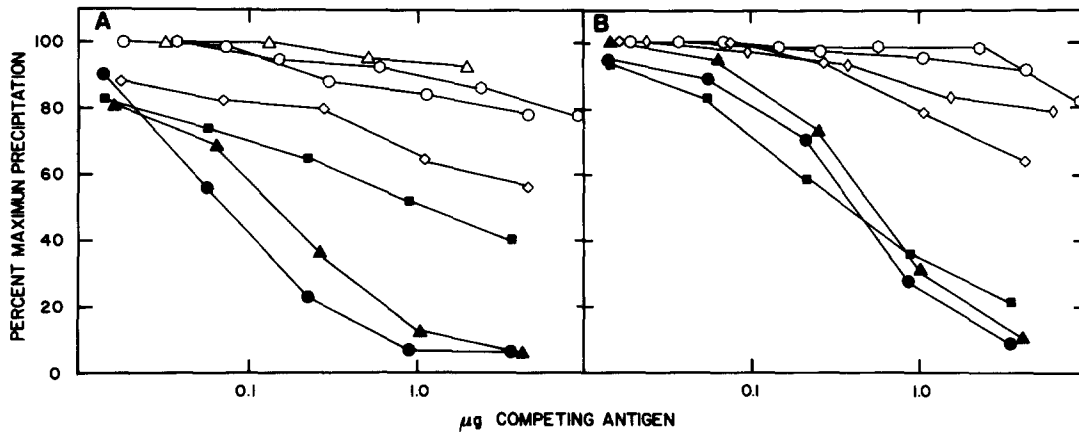


FIG. 4. Reactivities of mouse type-C viruses in type-specific MCF assays. Competition radioimmunoassays were carried out as described in Materials and Methods with (A)  $^{125}\text{I}$ -labeled Fr-MCF gp70 and a 1:400 dilution of anti-Fr-MCF serum; or (B)  $^{125}\text{I}$ -labeled Fr-MCF gp70 and a 1:400 dilution of anti-Mol-MCF serum. Competing viruses used in these assays were: Fr-MCF virus (●); Mol-MCF virus (▲); AKR-MCF virus (■); NZB xenotropic MuLV (◇); ATS xenotropic MuLV (◊); BALB:virus-2 xenotropic MuLV (○); FeLV/KiSV (Δ); and Friend, Moloney, or AKR ecotropic MuLV (○).

SFFV. We therefore developed a competition radioimmunoassay using Fr-MCF gp70 and antiserum prepared against Fr-MCF virus. Ecotropic, xenotropic, and MCF strains of MuLV were then tested as competing antigens in this assay. As shown in Fig. 4A, Fr-MCF virus competed very efficiently in this assay, as did the Mol-MCF virus. AKR MCF 247 virus competed to a lesser degree. A slight cross-reaction was seen with the NZB xenotropic virus, although another xenotropic virus, ATS-124, did not compete at all. Importantly, the ecotropic Fr-, Mol-, and AKR-MuLV did not significantly compete in this assay. Since the MCF viruses were purified from infected mink cells, FeLV/KiSV was included as a control and failed to cross-react in this assay. In studies not shown, Fr-MCF virus purified from infected FRE cells and Mol-MCF virus from BALB 3T3 cells also reacted fully in this assay.

Since the AKR MCF 247 virus competed poorly in the Fr-MCF-specific gp70 assay, we sought to broaden the assay in hopes that we would be better able to detect MCF-specific determinants on the AKR MCF gp70 without detecting the ecotropic or xenotropic gp70s. We therefore developed a competition radioimmunoassay utilizing Fr-MCF gp70 and antiserum to Mol-MCF virus. As shown in Fig. 4B, all three MCF viruses competed very well and were clearly distinguishable from the xenotropic and ecotropic MuLV. Importantly, NZB xenotropic virus competed only slightly in this assay, whereas two other xenotropic viruses, ATS-124 and BALB:virus-2, did not compete at all.

*Detection of a Cross-Reactive Antigen in Cells Infected with the SFFV.* Using molecular hybridization techniques, the SFFV has been shown to be a recombinant between F-MuLV<sub>eco</sub> and sequences related to the *env* gene of MCF and xenotropic viruses (6). The 5' end of the gag gene product, p15 and p12, has been detected in NRK cells nonproductively infected with SFFV (13, S. Aaronson, personal communication), but gp70 has not been detected in these cells. We therefore utilized several of the immunoassays that had been developed to detect Friend ecotropic or MCF gp70s to

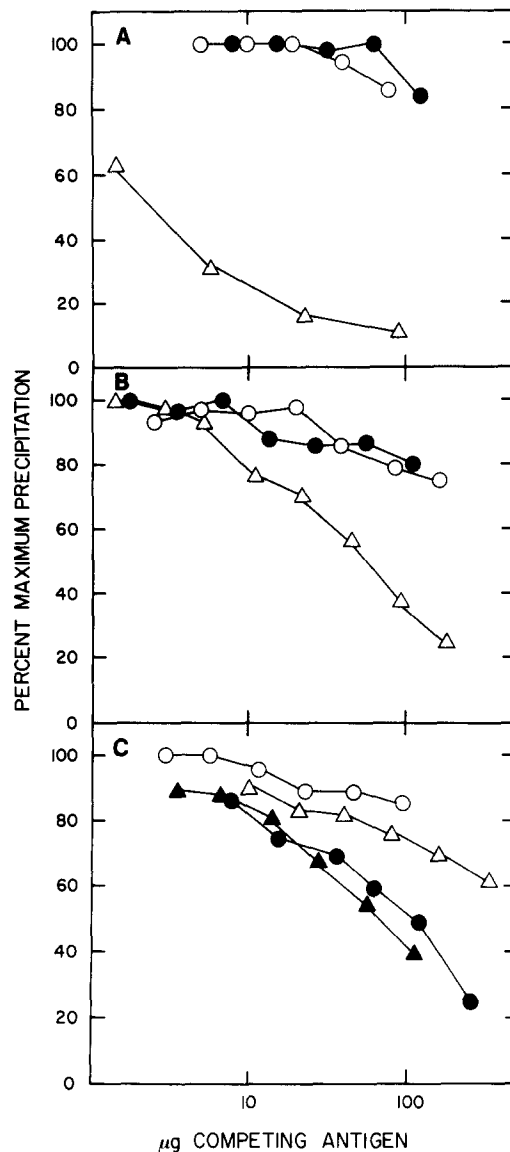


FIG. 5. Detection of an MCF-related antigen in rat cells infected with the SFFV. Competition radioimmunoassays were carried out as described in Materials and Methods utilizing (A)  $^{125}\text{I}$ -labeled F-MuLV<sub>eco</sub> gp70 and a 1:51,200 dilution of anti-Fr-MuLV<sub>eco</sub> serum; (B)  $^{125}\text{I}$ -labeled F-MuLV<sub>eco</sub> gp70 and a 1:160 dilution of anti-BALB:virus-2 serum; or (C)  $^{125}\text{I}$ -labeled Fr-MCF gp70 and a 1:400 dilution of anti-Mol-MCF serum. The following cell extracts were used as competing antigens in these assays: SFFV nonproducer NRK cells (●); SR-V-NRK cells (○); Fr-MuLV infected FRE cells (Δ); and FRE cells infected with SFFV and F-MuLV (▲).

determine if we could detect a cross-reactive antigen coded for by the SFFV genome. Using  $^{125}\text{I}$ -labeled F-MuLV gp70 in either a type-specific ecotropic assay with anti-Fr-MuLV<sub>eco</sub> serum (Fig. 5A) or a group-specific assay with anti-xenotropic BALB:virus-2 serum (Fig. 5B), F-MuLV-infected cells competed quite efficiently, whereas the NRK cells expressing SFFV competed no better than uninfected NRK

cells. However, when Fr-MCF gp70 and anti-Mol-MCF serum were utilized in a competition radioimmunoassay, extracts of the SFFV nonproducer NRK cells were clearly competitive compared to extracts of uninfected NRK cells (Fig. 5C). Another rat cell line (FRE) infected with both SFFV and F-MuLV was also able to compete much more efficiently in this assay than rat cells infected with F-MuLV alone. We can therefore conclude that the SFFV genome codes for an antigen which cross-reacts with Fr-MCF gp70 in an immunoassay which clearly distinguishes MCF gp70s from those of both ecotropic and xenotropic MuLV. In other studies (data not included), the reactivity from the SFFV-infected NRK cell could be purified as a discrete peak on a guanidine hydrochloride-agarose column eluting in the mol wt range of 50–55,000 daltons. No such reactivity was detected from an extract of NRK cells similarly processed on this column.

### Discussion

The current studies were designed to utilize a helper-independent MCF strain of Friend virus, which was shown to be an *env* gene recombinant between ecotropic and xenotropic MuLV, to investigate the proteins which might be coded for by a replication-defective recombinant virus, the SFFV. The results demonstrate that: (a) using the Fr-MCF gp70 and antisera to either Fr-MCF or Mol-MCF viruses, competition immunoassays can be developed which can distinguish MCF viruses from ecotropic viruses derived from Friend, Moloney, or AKR murine type-C viruses as well as from known helper-independent xenotropic viruses; and (b) such assays are able to measure a previously undetectable antigen coded for by the Friend strain of SFFV which cross-reacts with the MCF gp70s.

Immunological characterization of the Fr-MCF gp70 using antisera made against Fr-MuLV<sub>eco</sub> and Mol-MuLV<sub>eco</sub> demonstrates that, whereas the Fr-MCF gp70 has retained group-specific and interspecies-specific gp70 determinants, it has lost type-specific determinants of the Friend ecotropic type-C virus. At the same time, the gp70 of this recombinant virus derived from F-MuLV has acquired type-specific MCF determinants which are present on MCF strains of MuLV derived from three strains of ecotropic type-C viruses: Friend, Moloney, and AKR.

After studying by immunoassay the relationship between the type-specific MCF gp70 determinants and three isolates of xenotropic viruses, it can be concluded that MCF-specific determinants are not contained in the gp70s of BALB:virus-2 and ATS-124, although a weakly cross-reactive protein is detected in an NZB-MuLV. Since previous hybridization studies had shown that cDNA<sub>SFFV</sub>, a nucleic acid probe for the non-F-MuLV portion of SFFV, hybridizes with sequences in each of these xenotropic viruses (5), the MCF-specific immunoassay is apparently more discriminating than hybridization with cDNA<sub>SFFV</sub>.

Since the MCF gp70 assay can distinguish the MCF viruses from the helper-independent ecotropic and xenotropic viruses, the question of the origin of the MCF immunological determinant(s) coded for by MCF viruses and SFFV is raised. Several conceivable explanations exist. MCF-specific antigenic sites might be generated by a recombinational event involving specific primary amino acid sequence changes or conformational changes in the gp70 polypeptides of the MCF viruses and SFFV. This explanation is supported by the fact that the MCF class of viruses which arise by recombination between ecotropic and xenotropic viruses exhibits a unique biologic



property (mink cell focus induction) which is not a property of either xenotropic or ecotropic virus alone or in phenotypic mixtures (1, 14). Alternatively, the MCF-specific antigenic determinants might be derived from a distinct class of murine xenotropic viruses (possibly replication-defective) which are partially related by nucleic acid hybridization studies to known strains of xenotropic virus, but can be distinguished in a specific immunoassay such as we have described. Further experiments will be required to decide between these possibilities as well as to determine whether the MCF-specific determinants are protein or carbohydrate.

The ability to detect a cross-reactive protein in rat cells nonproductively infected with SFFV using this MCF assay supports earlier hybridization studies which indicated that SFFV arose as a result of recombination between F-MuLV<sub>eco</sub> and sequences related to the *env* gene of a xenotropic virus (5, 6). Furthermore, these results strengthen the hypothesis that the generation of SFFV might have been the result of a recombinational event similar to that giving rise to MCF-MuLV (3, 5, 6), since SFFV not only contains genetic sequences related to the xenotropic *env* genes in MCF viruses, but also codes for an antigen in common with MCF virus *env* gene products.

### Summary

We have isolated the gp70 of a helper-independent strain of a Friend mink cell focus-inducing (MCF) virus, Fr-MCF-1. This recombinant virus, like the previously described AKR-MCF viruses, has been shown by both biological and biochemical means to be an envelope gene recombinant between Friend murine leukemia virus (F-MuLV) and a mouse xenotropic virus. Utilizing <sup>125</sup>I-labeled Fr-MCF-1 gp70 and antiserum prepared against an MCF strain of Moloney type-C virus (Mol-MCF<sub>83</sub>), we have developed a radioimmunoassay which detects immunological determinant(s) contained in the gp70s of MCF viruses derived from F-MuLV, Mol-MuLV, and AKR-MuLV. This MCF determinant(s) is not detected in the ecotropic parents of each of these MCF viruses, nor in helper-independent murine xenotropic viruses derived from Swiss or BALB/c mice. A protein partially cross-reactive with the MCF gp70 determinant(s) is detected in a replicating xenotropic virus derived from NZB mice. Utilizing this MCF gp70 specific immunoassay, we can detect a cross-reacting gene product coded for by the Friend strain of the spleen focus-forming virus (SFFV) in rat fibroblasts nonproductively infected with SFFV. The results support earlier molecular hybridization studies which indicated that the genome of SFFV contains genetic information derived from both F-MuLV and xenotropic virus, and that the xenotropic-related sequences in SFFV are highly related to those found in MCF murine type-C viruses.

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