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Immunology

Characterization of a feline sarcoma virus-coded antigen (FOCMA-S) by radioimmunoassay

(viral transformation/tumor antigen/viral-coded polyprotein)

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ABSTRACT **A radioimmunoassay has been developed that detects a unique antigen encoded by the genome of the feline sarcoma virus (FeSV). Pseudotype viral particles containing an FeSV-specific polyprotein (p85) were used both as a source of antigen and to prepare specific antisera in rabbits. Because p85 contains antigens related to two structural proteins (p15 and p12) of feline leukemia virus (FeLV), antibodies directed to these were adsorbed with purified FeLV proteins. The adsorbed rabbit antiserum bound to antigenic determinants (designated FOCMA-S) which are also present in p85 and reacted specifically in immunofluorescence tests with rat cells transformed by FeSV and with FOCMA-positive cat lymphoid tumor cells. Competition assays detect FOCMA-S in pseudotype type C viruses rescued from FeSV-transformed mink and rat cells but not in heterologous type C helper viruses or in FeLV. A crossreactive antigen was also detected in pseudotypes of Kirsten sarcoma virus. The assay permits the quantitative measurement of an FeSV-coded protein whose expression is associated with viral transformation.**

Antibodies to the feline oncornavirus-associated cell membrane antigen (FOCMA) protect cats infected with feline type C viruses from developing virus-induced tumors (1–4). FOCMA is detected on membranes of cat fibrosarcomas induced by feline sarcoma virus (FeSV) and on lymphoid tumor cells induced by feline leukemia virus (FeLV) (1, 4–6). A similar antigen is expressed on "nonproducer" mink and rat cells transformed by FeSV (7). Although the antigens detected in lymphoid tumors in FeLV-infected cats and in FeSV-transformed fibroblasts are completely crossreactive (6, 8), some antigenic determinants could be unique to each system. We therefore propose that the terms FOCMA-L and FOCMA-S be used for the antigens induced by FeLV and FeSV, respectively.

Pseudotype virions obtained by rescue of the FeSV genome from nonproducer cells by using different heterologous type C viruses contain FOCMA-S as well as two FeLV-related structural protein antigens, p15 and p12 (8). FOCMA-S, p15, and p12 cochromatograph as phosphorylated molecules of 85,000 molecular weight (p85) and coprecipitate with antisera to FeLV or to FOCMA, suggesting that the three antigens are covalently linked in p85. Because FeLV p15 and p12 do not react with cat antibodies to FOCMA, FOCMA-S appears to reside in a unique domain of p85 that lacks p15 and p12 amino acid sequences. FeSV-transformed mink cells also synthesize molecules ($p \sim 90$) with FOCMA-S, p15, and p12 antigenic determinants; p90 is processed into two separate species containing FOCMA-S (p60) and the two linked FeLV p15 and p12 polypeptides (p25) (6, 9).

We have now developed a radioimmunoassay for FOCMA-S using reagents generated entirely outside of a cat cell system. FeSV pseudotype virions were used to prepare rabbit antisera directed toward FOCMA-S and also as a source of FeSV p85 protein. With appropriately adsorbed antisera, this assay detects FOCMA-S but not the major structural proteins of FeLV.

MATERIALS AND METHODS

Cells and Viruses. Mv1Lu mink cells were used to prepare nonproducer clones (64F1CL10, 64J1) transformed by FeSV and Kirsten sarcoma virus (KiSV), respectively; rat cell nonproducers (F3-NRKCL2, KNRK) transformed by FeSV and KiSV were similarly derived (10). Moloney sarcoma virus (M-MuSV; S^+L^- strain) nonproducer mink cells were obtained from Paul Peebles. Isopycnically banded viral pseudotype particles were purified after rescue of sarcoma viral genomes as previously described; helper type C viruses included baboon virus (M7), two mouse xenotropic viruses (AT-124; BALB/c X-tropic virus), the endogenous cat virus (RD-114), and the simian sarcoma-associated virus (SSAV) (8). An endogenous mink virus (MiLV) was grown in dog cells (11). FeLV (F422; Rickard strain) and FeSV (FeLV) (Gardner–Arnstein) were obtained from the Resources Office of the National Cancer Institute. Mouse 3T3 cells producing M-MuSV (MuLV) were from Peter Fischinger. FeSV, M-MuSV, and KiSV pseudotypes and FeSV (FeLV) contained about 10^5 focus-forming units (FFU) per ml; M-MuSV (MuLV) contained 10^8 FFU per ml.

Biochemical Techniques. Gel filtration in Bio-Gel A5-M containing 6 M guanidine hydrochloride (Gdn HCl) was performed as described (8, 12) and was used to purify FeSV p85 as well as the major structural proteins (p30, p15, and p12) of FeLV and M7 baboon virus. The gp70 proteins of M7 and FeLV were purified by sequential chromatography on phosphocellulose and Sephadex G-100 as described by Strand and August (13). Electrophoresis of dissolved immune precipitates was performed in cylindrical 7.5% polyacrylamide gels containing sodium dodecyl sulfate ($NaDodSO_4$) (14). Proteins were measured by the method of Lowry *et al.* (15).

Serum Adsorption and Immune Precipitation. Fractions from GdnHCl-containing agarose columns containing p85 were radiolabeled with ^{125}I to high specific activity ($\sim 1.5\ \mu Ci/\mu g$) (16). A rabbit antiserum was raised toward Triton X-100/ether-disrupted FeSV (M7) virus rescued from transformed mink cells (17). The serum was diluted with an equal volume of fetal calf serum and adsorbed with disrupted, lyophilized viral proteins or purified FeLV p15 and p12 as indicated in Table 1. All adsorptions were performed for 60 min at 37° and for 2 hr at 4° with 1 mg of lyophilized virus per 0.2 ml of rabbit

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Abbreviations: FOCMA, feline oncornavirus-associated cell membrane antigen; FeSV, feline sarcoma virus; FeLV, feline leukemia virus; KiSV, Kirsten sarcoma virus; M-MuSV, Moloney murine sarcoma virus; FFU, focus-forming units; GdnHCl, guanidine hydrochloride; $NaDodSO_4$, sodium dodecyl sulfate; RIA, radioimmunoassay; SSAV, simian sarcoma-associated virus; RSV, Rous sarcoma virus; MiLV, endogenous mink virus.

antiserum or 50 μg of purified p15 or p12 protein. Adsorbed sera were clarified by centrifugation at 10,000 × *g* for 10 min. Limiting concentrations of antisera (2.5–5 μl) were allowed to react in solution with an excess (~1 × 10^6 cpm of ^{125}I-labeled p85) of labeled antigen sufficient to saturate all antibody combining sites. Reaction tubes (0.5 ml) contained 0.01 M phosphate, pH 7.5/0.15 M NaCl/0.1% Triton X-100. After incubation for 30 min at 37°, an excess of sheep antiserum to rabbit IgG was added, and incubation was continued for 30 min at 37° and for 2 hr at 4°. Immune precipitates were collected by centrifugation, washed five times in buffer, and dissolved in 0.2 ml of 0.1 M phosphate, pH 7.2/0.2 M 2-mercaptoethanol/0.5 M urea/1% $NaDodSO_4$/0.05% bromophenol blue/10% (vol/vol) glycerol. Radioactivity in 10-μl aliquots was determined, and 30-μl portions of the same samples were applied to gels.

Immunofluorescence Assay. Adsorbed rabbit antisera were assayed for anti-FOCMA activity by using F3-NRKCL2 and cat lymphoid tumor (FL74) cells as targets (7).

Radioimmunoassays. Radioimmunoassays for FeLV p15 and p12 proteins and for the gp70, p30, p15, and p12 proteins of the M7 baboon virus were performed as described (8, 11, 17). A radioimmunoassay for FOCMA-S was designed using rabbit antiserum to FeSV (M7) adsorbed with fetal calf serum, disrupted and lyophilized M7 and MiLV virions, and FeLV p15 and p12 proteins. Adsorbed sera contained no residual antibody activity to M7 or FeLV proteins, as determined by sensitive radioimmunotitration tests (17) performed with the respective ^{125}I-labeled viral proteins. All reaction tubes contained 0.5 ml of 0.02 M phosphate, pH 7.2/0.15 M NaCl/0.1% Triton X-100/5 mM EDTA, 2 μl of adsorbed rabbit antiserum, and 30,000 cpm of ^{125}I-labeled p85 protein. Under these conditions, approximately 5000 cpm of tracer antigen was bound in immune complexes in the absence of competing proteins. Assays were performed with a double-antibody method to precipitate immune complexes (18).

RESULTS

FeSV (M7) virions released from mink cells were disrupted with detergent and an aliquot was radiolabeled with ^{125}I. Denatured, labeled virions were chromatographed in the presence of 6 M GdnHCl, and the positions of viral proteins were determined by the distribution of radioactivity in the column effluent. Six major peaks corresponding to the column void volume and to proteins of 85,000, 70,000, 30,000, 15,000, and 12,000 molecular weight were resolved (Fig. 1). Four proteins (gp70, p30, p15, and p12) are detected in the M7 helper virus alone; p85 has only been found in FeSV pseudotype preparations (8).

Viral proteins from the column were pooled, concentrated, and tested in competitive radioimmunoassays (RIAs) for each of the M7 viral structural proteins. These studies confirmed the identity of M7 gp70, p30, p15, and p12 proteins and showed that <10% of the total protein in the p85 fraction was M7 gp70. All fractions from the column were also tested in RIAs for FeLV p15 and p12 proteins. In agreement with previous results (8), >90% of the FeLV p15 and p12 activities were detected in the p85 fraction with the remainder distributed equally between the void volume and gp70 fractions; no FeLV p15 or p12 was detected in positions corresponding to authentic p15 or p12 proteins.

Fig. 2 shows a representative RIA for FeLV p15 protein. A 20% displacement of the labeled "tracer" protein was obtained with 0.7 ng of competing p15 protein. FeSV (M7) virions competed in this assay as did the p85 fraction obtained from the column shown in Fig. 1. Comparison of the latter two

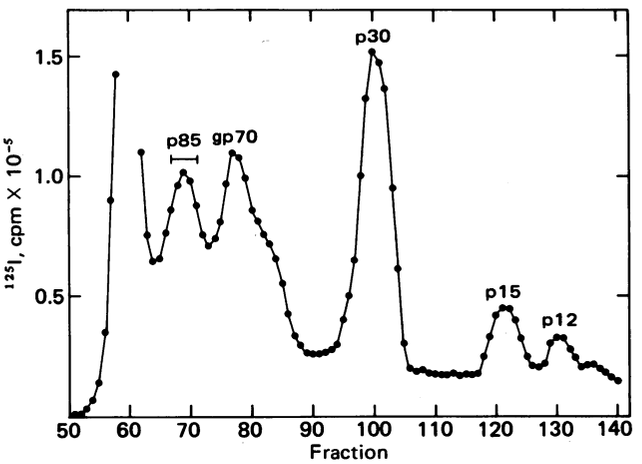

FIG. 1. Gel filtration of FeSV (M7) virus in Bio-Gel A5-M containing 6 M GdnHCl. Disrupted virus radiolabeled with ^{125}I was mixed with unlabeled virus prior to application to the column. The brackets indicate pooled fractions containing p85. The positions of baboon viral proteins are also indicated. The M7 p10 protein is poorly radiolabeled by this method.

competition curves shows that p85 was purified approximately 50-fold by gel filtration. A 20% displacement of the tracer p15 protein was obtained with 10.5 ng of p85 protein. Assuming that there is 1 mol of p15 per mol of p85 (6, 8), we estimate that 38% of the protein in this fraction is authentic p85 (see below). Similar estimates for purity of p85 (34%) were obtained by using an FeLV p12 assay.

Protein in the p85 fraction was relabeled with ^{125}I to high specific activity (~1.5 μCi/μg) and was precipitated with a rabbit antiserum raised against the homologous FeSV (M7) virus grown in mink cells. For these experiments, an "antigen excess" method was used. Limiting concentrations of preadsorbed antisera were allowed to react with quantities of ^{125}I-labeled p85 that saturated all antibody combining sites. After formation of soluble immune complexes, an antiserum to purified rabbit IgG was added and the resulting precipitates were assayed for radioactivity. These experiments permit determination of the specificities of different antibodies in a serum preparation independent of their respective titers.

Table 1 shows the results of precipitation studies using various adsorbed antisera and the results of immunofluorescence assays

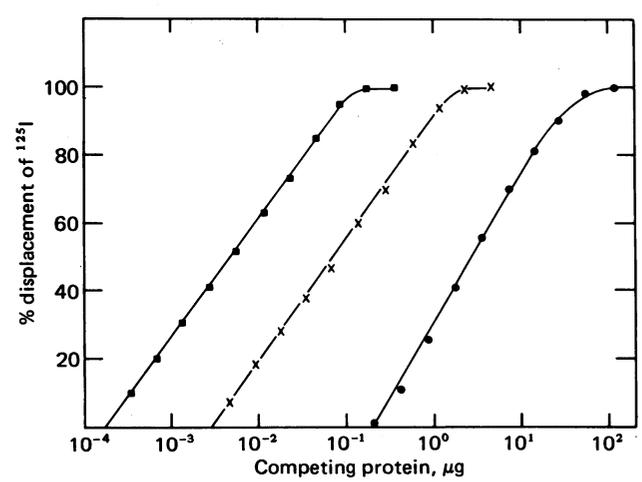

FIG. 2. Radioimmunoassay for FeLV p15. ■, FeLV p15 protein; X, p85 protein from FeSV (M7) virions; ●, FeSV (M7) virus.

Table 1. Characterization of rabbit antiserum to FeSV (M7)

Antiserum adsorbed with	^{125}I-p85 cpm precipitated	Immunofluorescence titer		
		F3NRK	NRK	FL74
None	10,222	64	2–4	64
Fetal calf serum	5,124	64	2–4	32–64
+ mink cells	5,086	64	2–4	32–64
+ MiLV	4,859	32–64	4	32
+ M7	4,468	32–64	4	32–64
+ M7 + MiLV	4,501	32–64	4	32
+ M7 + MiLV + FeLV p15	3,161	32–64	4	32
+ M7 + MiLV + FeLV p15/p12	3,015	32–64	4	32
+ M-MuSV (M7)	4,206	32–64	2	32
+ KiSV (M7)	3,621	16	2	8–16
+ FeSV (M7)	732	2	2–4	2
Normal Serum	571	1	1	1

Aliquots (0.2 ml) of rabbit antiserum to FeSV (M7) were adsorbed with 0.2 ml of fetal calf serum, with 1 mg of lyophilized viral protein, or with 50 μg of lyophilized FeLV p15 or p12 proteins as indicated. Binding reactions were initiated in 0.5-ml solutions using 2.5 μl of unadsorbed sera or 5 μl of adsorbed sera and 1×10^6 cpm of ^{125}I-labeled p85 protein. Following precipitation of immune complexes with antiserum to rabbit IgG, precipitates were dissolved (0.2 ml) and radioactivity in 10-μl portions was determined as shown. The same sera were tested for FOCMA-S and FOCMA-L immunofluorescence by using FeSV-transformed rat (F3NRK) and cat lymphoid tumor (FL74) cells, respectively.

for both FOCMA-S and FOCMA-L. Without adsorption, a limiting concentration of rabbit antiserum to FeSV (M7) precipitated approximately 10,000 cpm of labeled protein from the ^{125}I-labeled p85 preparation. Only 50% of the radioactivity was precipitated after adsorption of the serum with an excess of fetal calf serum, indicating that about half of the antibodies reacted with ^{125}I-labeled molecules derived from calf serum components contaminating the p85 fraction. Further adsorption of the antiserum with extracts of mink cells or with the endogenous mink virus occasionally released from these cells (11) did not significantly decrease the amount of radioactivity precipitated. A slight reduction (~5%) in precipitated radioactivity was detected after adsorption with an excess of M7 helper virus, consistent with the presence of low levels of M7 gp70 in the p85 fraction (see above). By contrast, adsorption of the serum with FeSV (M7) virions decreased the quantity of precipitable radioactivity to virtually background levels, indicating the presence of FeSV-specific antibodies in this serum. In general agreement with the data shown in Fig. 2, these results suggested that the p85 preparation contained substantial quantities of authentic p85, that most contaminating protein was derived from calf serum components, and that only a small percentage of the protein in the fraction was derived from the helper baboon virus.

Of the antibodies directed to p85, we anticipated that some would react with FeLV-related p15 and p12 antigenic determinants and others would react with FOCMA-S. Further absorptions were therefore performed using purified FeLV p15 and p12 proteins, and removal of antibodies to p15 and p12 was confirmed by sensitive radioimmunotitration experiments using ^{125}I-labeled FeLV p15 and p12 proteins. Table 1 shows that only some of these antibodies were directed toward p15 and p12 antigenic determinants in p85 and that residual antibodies still reacted to the FeSV protein, presumably to determinants within the FOCMA-S moiety. Thus, antibodies to FOCMA-S do not react with FeLV p15 or p12 even though the three antigens are associated in p85.

In agreement with the immunoprecipitation data, all of the adsorbed sera, with the exception of that adsorbed with FeSV (M7), reacted to FOCMA-S determinants when tested by immunofluorescence with FeSV-transformed rat cells (Table 1). These sera also crossreacted at similar dilutions with FOCMA-L determinants on FL74 cat lymphoid tumor cells. Antibodies to FOCMA were not adsorbed with M-MuSV (M7) pseudotype virions derived from mink cells. However, adsorption with a KiSV (M7) pseudotype partially decreased the immunofluorescence titers with both F3-NRK and FL74 cells, suggesting that KiSV pseudotypes contain a crossreactive antigen.

Portions of the same dissolved immunoprecipitates were electrophoresed on polyacrylamide gels containing $NaDodSO_4$. Fig. 3 shows that the antiserum adsorbed with fetal calf serum precipitated predominantly p85 although a shoulder was detected in the region of the gel corresponding to the position of baboon viral gp70. The shoulder was not detected with antiserum adsorbed with M7 virus and FeLV p15 and p12. As expected, there was a reduction in the magnitude of the major radioactive peak when this serum was used (see Table 1). By contrast, normal rabbit serum or immune rabbit serum adsorbed with FeSV (M7) failed to precipitate ^{125}I-labeled p85.

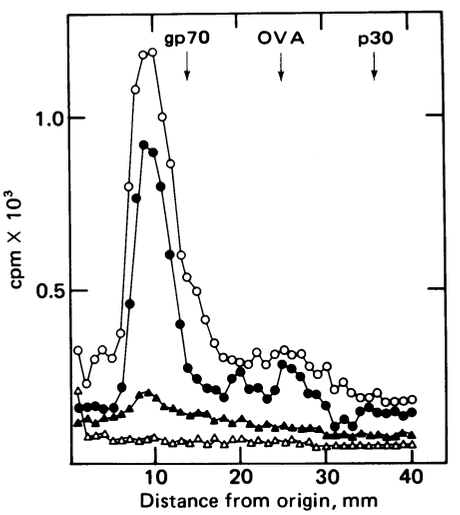

FIG. 3. Electrophoresis of dissolved immunoprecipitates in 7.5% polyacrylamide gels containing 0.1% $NaDodSO_4$ and 0.5 M urea. The samples were derived from the experiment shown in Table 1 and represent application of 0.03-ml aliquots from 0.2-ml samples. The positions of ^{125}I-labeled marker proteins (M7 gp70, ovalbumin, and M7 p30 protein) run on parallel gels are indicated. ○, Rabbit antiserum to FeSV (M7) adsorbed with fetal calf serum; ●, rabbit anti-FeSV (M7) adsorbed with fetal calf serum, M7 virus, and FeLV p15 and p12; (▲) rabbit anti-FeSV (M7) adsorbed with fetal calf serum and FeSV (M7) virus; △, normal rabbit serum.

A radioimmunoassay was developed for the FeSV-specific gene product (FOCMA-S) using ^{125}I-labeled p85 and immune rabbit serum to FeSV (M7). The antiserum was adsorbed with fetal calf serum, M7 helper virus, and MiLV and also with FeLV p15 and p12 proteins to ensure that the remaining antibodies would be directed only toward FOCMA-S antigenic determinants. Titration of ^{125}I-labeled p85 with "an excess" of adsorbed rabbit antiserum showed that a maximum of 30% of the labeled protein could be precipitated, indicating that approximately one-third of the radioactivity represented FOCMA-S determinants. Fig. 4*A* shows that FeLV gp70, p30, p15, and p12 did not compete for the ^{125}I-labeled p85 tracer antigen but the unlabeled p85 fraction competed efficiently. This confirmed that antibodies reacting with ^{125}I-labeled p85 did not bind to p15 or p12 antigenic determinants and also showed that FeLV p30 and gp70 antigens could not be detected in p85 (cf. refs. 6, 8, and 9). Fig. 4*B* shows that four FeSV pseudotypes competed in the RIA regardless of the helper virus used in rescue (M7, RD-114, AT-124, or BALB/c X-tropic) or of the host nonproducer cell (mink or rat). Neither FeLV grown in cat or dog fibroblasts nor the helper viruses alone competed significantly.

The same FeSV pseudotypes were tested in RIAs for the p15 (Fig. 4*C*) and p12 (Fig. 4*D*) proteins of FeLV. Each of these assays fails to detect the various helper viruses (11). The p15 and p12 assays are more sensitive than the assay of FOCMA-S because each of the ^{125}I-tracer proteins can be labeled to higher specific activity (~5–10 μCi/μg versus ~1.5 μCi/μg for p85) without loss of antigenic reactivity. Thus, a 20% displacement in the RIA for p15 can be obtained with 0.7 ng of purified p15 protein (Fig. 2); a similar degree of competition in the p12 assay is obtained with 2.3 ng of purified p12 protein. By contrast, a 20% displacement in the RIA for FOCMA-S required 42 ng of competing protein from the p85 fraction (Fig. 4*A*); this corresponds to approximately 12.6 ng of FOCMA-S antigen, assuming that 30% of the protein in the p85 fraction represents these determinants (see above). Comparison of the competition curves obtained with p85 protein in the RIA for p15 (Fig. 2) and FOCMA-S (Fig. 4*A*) shows the expected increased sensitivity of the p15 assay in scoring p15 antigenic determinants in p85 molecules. For each FeSV pseudotype preparation tested, there was a concordance in the expression of FOCMA-S, p15, and p12 antigens, showing that all such pseudotypes contain the three FeSV-coded antigens.

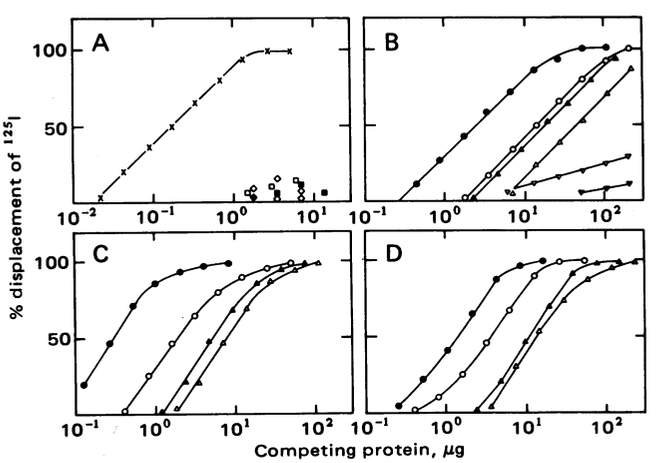

FIG. 4. Radioimmunoassays for FOCMA-S (*A* and *B*), FeLV p15 (*C*), and FeLV p12 (*D*). X, FeSV (M7) p85; ■, FeLV p15; □, FeLV p12; ◊, FeLV gp70; ●, FeSV (M7) from mink cells; ○, FeSV (AT-124) from rat cells; ▲, FeSV (RD-114) from mink cells; △, FeSV (BALB/c X-tropic) from rat cells; ▼, M7 virus grown in human cells; ▽, FeLV (Rickard strain) or FeLV (Gardner–Arnstein) grown in cat or dog fibroblasts, respectively. The FeLV preparations contained approximately 280 μg of p30 antigen per mg of competing protein.

Fig. 5 shows results obtained with several other viruses in the RIA for FOCMA-S. Cat cell-grown FeSV (FeLV) with a focus-forming titer similar to that of the various FeSV pseudotypes contained relatively low levels of FOCMA-S, suggesting that packaging of the antigen may be less efficient in FeSV stocks produced with the homologous helper virus. KiSV (M7) from mink cells and KiSV (AT-124) from rat cells also scored in the assay. The decreased slopes of the competition curves suggest that KiSV codes for a different antigen which crossreacts with FOCMA-S. By contrast, high-titer M-MuSV (MuLV) grown in mouse cells and SSAV grown in rat NRK cells failed to compete. M-MuSV (M7) pseudotypes rescued from mink nonproducer cells also did not score in this test.

DISCUSSION

The expression of FOCMA in FeSV-transformed rat and mink nonproducer cells provides evidence that this antigen is encoded by a feline type C viral gene (7). The presence of FOCMA-S in FeSV pseudotype particles has facilitated the development of a radioimmunoassay for an FeSV gene product which uses rabbit antiserum and protein derived from viral particles produced by nonfeline cells. Thus, it is now possible to assay for FOCMA-S with reagents generated completely outside of the feline system. Although the polyprotein (p85) detected in pseudotype virions contains p15 and p12 antigenic determinants, antibodies to these can be adsorbed. Hence, the RIA for FOCMA-S does not detect the major viral structural protein antigens found in p85.

The RIA detects FeSV pseudotypes but not their helper viruses or FeLV itself. Of the known FeLV structural proteins, purified viral gp70, p30, p15, and p12 were directly tested and did not compete for FOCMA-S determinants in the p85 tracer antigen. The absence of competition when FeLV was used further suggests that FeLV p10 lacks FOCMA-S determinants because the competing FeLV preparations were of very high titer and should have contained high concentrations of this protein. However, it is difficult to exclude the presence of FOCMA-S determinants in viral reverse transcriptase because the enzyme represents only a small fraction of the total virion

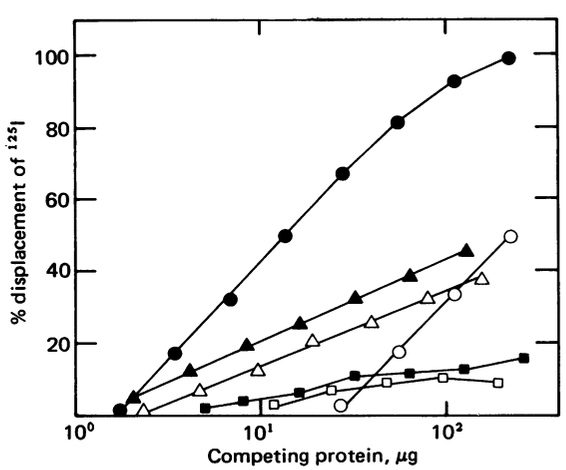

FIG. 5. Radioimmunoassay for FOCMA-S. ●, FeSV (AT-124) from rat cells; ○, FeSV (FeLV) from cat cells; ▲, KiSV (M7) from mink cells; △, KiSV (AT-124) from rat cells; ■, M-MuSV (MuLV) from mouse cells; □, SSAV grown in rat cells.

protein. High-titer antisera raised to the polymerase of FeLV and adsorbed with p15 and p12 proteins do not react with ^{125}I-labeled p85; nor do rabbit antisera to p85 inhibit FeLV polymerase activity (unpublished data). Thus, the results suggest that the RIA detects only a sarcoma virus-specific antigen.

Although FOCMA-S was readily scored in FeSV pseudotypes, it was marginally detected in FeSV (FeLV) preparations of equivalent focus-forming titer grown in cat cells. This is consistent with the failure to detect significant quantities of ^{32}P-labeled p85 molecules in such stocks (8). In cells, both p85 and its p60 derivative contain FOCMA-S determinants (6) but only p85 is detected in rescued pseudotype virions (8). Thus, the specificity for packaging the polyprotein appears to reside in the p15–12 moiety. The reduced concentration of p85 in FeSV (FeLV) may then reflect an increased rate of processing in cat cells infected with the homologous helper virus.

An antigen distinct from FOCMA-S was detected in KiSV pseudotypes rescued from rat or mink cells. The decreased slopes of the competition curves in the RIA (Fig. 5) and the partial adsorption of anti-FOCMA sera with KiSV (M7) (Table 1) both suggest that KiSV codes for a crossreactive antigen. Both rabbit and cat antisera to FOCMA exhibit low-titer immunofluorescence with KiSV-transformed mink but not rat nonproducer cells (unpublished data). Although the differences between these cell systems are as yet unexplained, the results could reflect quantitative differences in antigen expression similar to those seen with FeSV-transformed mink and rat nonproducer cells (6). By contrast, a similar antigen was not detected in a high-titer M-MuSV (MuLV) preparation, in M-MuSV (M7) pseudotypes rescued from mink cells, or in SSAV grown in rat cells. The differences obtained with M-MuSV and KiSV could reflect the different rodent origins of these viruses (mouse and rat, respectively) (19) and the homology of rat and feline type C viral genes (20).

The FeLV genome contains three genes designated *gag*, *pol*, and *env*. The 5′ *gag* gene codes for a precursor (pr70^{gag}) of the low molecular weight viral proteins containing the polypeptide sequence NH_2-p15-p12-p30-p10-COOH (9, 21). By analogy to the murine system, low-frequency "read through" into the adjacent *pol* gene would produce a second precursor (pr200$^{gag\text{-}pol}$) containing pr70^{gag}, reverse transcriptase, and "extra" polypeptide sequences of 40,000–50,000 molecular weight (22). FeSV appears to contain an additional gene (*src*) which confers the ability to transform fibroblasts. If FOCMA-S were a product of the *src* gene, some mechanism would have to exist to permit continuous translation of *gag* (p15 and p12) and *src* gene products. The ability of FeLV to induce FOCMA-L in cat lymphoid tumor cells would then suggest that cat cells contain inducible *src* sequences that may have recombined with FeLV in the formation of FeSV. Alternatively, FeLV itself might code for a related antigen that does not get efficiently packaged into virions. For example, FOCMA could derive entirely from the 5′ end of the cat type C viral genome and could represent some aberrant product of pr200$^{gag\text{-}pol}$ formed secondarily to deletion of a portion of the *gag–pol* sequence. Thus, FeLV could give rise to replication-defective deletion variants coding for proteins analogous to FOCMA-S. The latter model would predict that sequences shared in common by FeSV and FeLV include those coding for FOCMA.

FOCMA-S appears to be analogous to polyproteins recently identified in cells infected with other replication-defective transforming avian (23) and mammalian (24) viruses. Each of these polyprotein products contains 5′-*gag* gene antigens as well as a moiety that, like FOCMA-S, appears to be unique to the transforming virus. Thus, the synthesis of proteins such as p85 may be a general property of replication-defective transforming viruses and is not restricted to the FeSV system. A protein (p60) has also been detected in cells transformed by Rous sarcoma virus (RSV), where its appearance is associated with transformation (25). This protein is not found in cells infected with transformation-defective RSV variants or in cells infected with temperature-sensitive transforming mutants and grown at the nonpermissive temperature. RSV p60 which, unlike FeSV p85, lacks *gag* gene-coded antigens and is not found in virions, is encoded by sequences contained in the 3′ one-third of RSV RNA and appears to represent a *src* gene product (26).

Antibodies to FOCMA are important in protecting cats against the development of virus-induced neoplastic disease (1–4). The purification of p85 should therefore facilitate studies of whether antibodies to FOCMA-S are of direct immunotherapeutic value. In addition, a quantitative assay for FOCMA should be useful in attempts to purify similar antigens from transformed cells and in characterizing these potential "transforming proteins."

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