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Immunology

Pseudotypes of feline sarcoma virus contain an 85,000-dalton protein with feline oncornavirus-associated cell membrane antigen (FOCMA) activity

(viral transformation/viral-coded antigens/tumor immunity)

CHARLES J. SHERR*, ARUP SEN*, GEORGE J. TODARO*, ANN SLISKI†, AND MAX ESSEX†

* Laboratory of Viral Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014; and † Department of Microbiology, Harvard School of Public Health, Boston, Massachusetts 02115

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ABSTRACT **Feline sarcoma virus (FeSV) rescued from transformed nonproducer mink or rat cells contains two FeSV-specific antigens (p15 and p12), and the feline oncornavirus-associated cell membrane antigen (FOCMA). All three antigens are helper virus-independent and are encoded by the FeSV genome. FOCMA, p15, and p12 antigens cochromatograph as phosphorylated molecules of 85,000 molecular weight (pp85), adsorb to immunoadsorbant columns prepared with antibodies to feline leukemia virus (FeLV), and are precipitated with antisera to FeLV or FOCMA. Antibodies to FOCMA can be adsorbed with fractions containing pp85 but not with FeLV proteins, including p15 and p12. Thus, a virus-coded tumor antigen which immunizes cats against tumors induced by feline type C viruses is packaged in FeSV particles and is linked to viral structural protein.**

The feline oncornavirus-associated cell membrane antigen (FOCMA) is found on cat cells transformed by feline leukemia (FeLV) or sarcoma (FeSV) viruses (1–3). Under experimental or field conditions, titers of antibodies to FOCMA in virus-exposed cats correlate inversely with the rate of tumor progression (1, 4–6), and cells expressing FOCMA can immunize animals against viral-induced leukemias and sarcomas (7, 8). Antibodies to FOCMA, then, are important in determining whether cats can successfully resist tumors caused by oncogenic feline type C viruses.

Adsorption of cat antisera reactive to FOCMA with disrupted FeLV (3, 9) or with two proteins (gp70 and p30) purified from virions (10) fails to reduce the anti-FOCMA titers. Surveys of cat sera have shown no concordance between titers of antibodies to FOCMA and those to viral gp70, p30 (3, 10), or reverse transcriptase (unpublished observations). Thus, FOCMA appears to be distinct from the major FeLV structural proteins.

"Nonproducer" mink cells transformed by FeSV also express FOCMA on their cell surfaces, suggesting that FOCMA is encoded by the feline sarcoma virus genome (11). In this report, we show that viral pseudotypes obtained from FeSV-transformed mink or rat nonproducer cells contain FOCMA as well as FeLV-related p15 and p12 antigens.

MATERIALS AND METHODS

Cells and Viruses. Mv1Lu mink cells (CCL 64) obtained from the American Type Culture Collection were used to prepare "nonproducer" clones transformed by FeSV (64F1 CL10, 64F3 CL7, and 64F2) or by Kirsten sarcoma virus (64J1) (12). A nonproducer rat cell clone (F3-NRK CL2) containing FeSV was similarly derived (12). Dog kidney cells (MSV/DK) transformed by Moloney [S^+L^-] mouse sarcoma virus (MSV) were obtained from Paul Peebles. Mink cells productively transformed with the HT-1 strain of Moloney MSV (13) were obtained by infection with MSV (M7) pseudotype stocks. Other lines included the human rhabdomyosarcoma A204 (14), canine thymus cells (FCf2Th), rat kidney cells (NRK) (15), and cat kidney cells (FEC).

Type C viruses included the endogenous baboon viruses M7 and M28 (16, 17), the simian sarcoma-associated virus (SSAV) (18), the xenotropic mouse virus AT-124 (19), the endogenous cat virus RD-114 (20), the endogenous mink virus MiLV (21), and the Rickard strain (F422) of FeLV (22). Isopycnically banded (ρ = 1.16 g/ml in sucrose) virions containing FeSV were obtained by infection of nonproducer mink or rat cells with helper viruses (12).

Radioimmunoassays. Purification of viral p30, p15, and p12 proteins, preparation of antisera, and techniques for radioimmunoassays were as described (23–25).

Gel Filtration. Viruses disrupted with detergent were filtered on 90 × 1.5 cm columns of Sephadex G-200 (23, 26). Fractions were concentrated by lyophilization and assayed for viral proteins by radioimmunoassay. Gel filtration in Bio-Gel A5M containing 6 M guanidine hydrochloride and 20 mM dithiothreitol was performed as described (25). Cells producing virus were labeled with ^{32}P (27). Extracellular virions were concentrated, disrupted, labeled with ^{125}I (28), and denatured in the presence of 0.1 M 2-mercaptoethanol (25). Regions of the effluent containing viral proteins were dialyzed and lyophilized (25).

Preparation of Immunoadsorbant Columns. FeLV (40 mg of protein) was disrupted with detergent, filtered over Sephadex G-25, and lyophilized. Viral protein suspended in 0.2 M Na citrate, pH 6.5, was conjugated to CNBr-activated Sepharose 4B (29); 70 mg of CNBr was used per ml Sepharose, and protein (4 mg/ml of Sepharose) was conjugated for 18 hr at 4°. Goat antiserum to Tween/ether-disrupted FeLV was fractionated with 50% ammonium sulfate. The precipitate was suspended and dialyzed against 0.1 M Tris buffer, pH 8.0, containing 0.1 M NaCl and adsorbed to the column containing FeLV proteins. Antiviral antibodies were eluted with 0.5 M acetic acid, neutralized, dialyzed, and lyophilized. Antibodies at a concentration of 8 mg/ml had radioimmunoassay titers (24) of 15,000,000 against FeLV p30, 80,000 against FeLV p15, and 7000 against FeLV p12 proteins. An immunoadsorbant containing purified antiviral antibodies was prepared as described above.

Serum Adsorptions and Immunofluorescence. Cat sera

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Abbreviations: FOCMA, feline oncornavirus-associated cell membrane antigen; FeLV, feline leukemia virus; FeSV, feline sarcoma virus; MSV, mouse sarcoma virus; SSAV, simian sarcoma-associated virus; NaDodSO$_4$, sodium dodecyl sulfate; KiSV, Kirsten sarcoma virus; MiLV, endogenous mink type C virus.

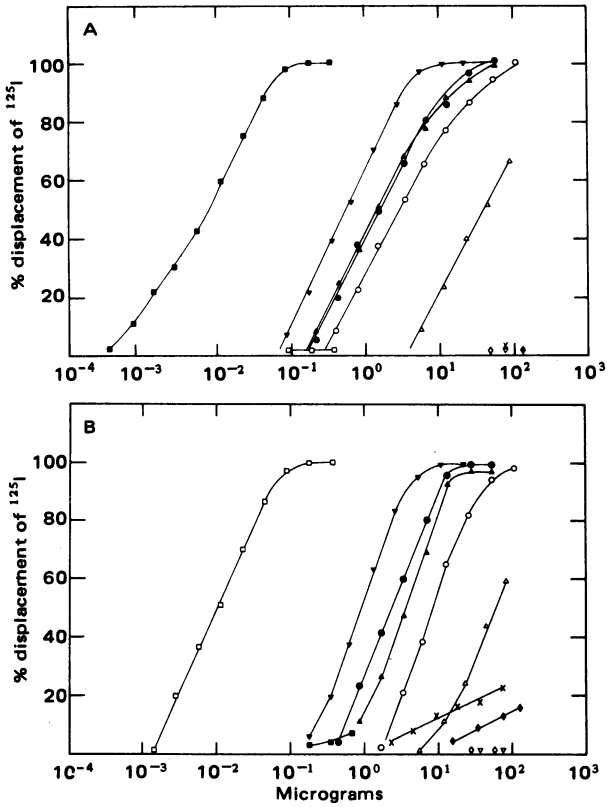

FIG. 1. Radioimmunoassays for FeLV p15 (*A*) and p12 (*B*) proteins. Symbols: ■, FeLV p15 protein; □, FeLV p12 protein; ▼, FeSV (M7) from 64F1 CL10 mink cells; ●, FeSV (M7) from 64F2 mink cells; ▲, FeSV (AT-124) from F3-NRK CL2 rat cells; ○, FeSV (AT-124) from 64F1 CL10 mink cells; △, FeSV (SSAV) from F3-NRK CL2 rat cells; X, KiSV (M7) from 64J1 mink cells; ◊, AT-124 grown in human cells; ▽, M7 grown in human cells; ♦, MiLV grown in dog cells.

containing antibodies to FOCMA were adsorbed with lyophilized proteins (see Table 2) for 30 min at 37° and for 18 hr at 4°. After centrifugation, serial dilutions were tested for anti-FOCMA reactivity (30) with 64F3 CL7 cells as targets (11).

Immune Precipitation and Electrophoresis. Fractions from guanidine hydrochloride-containing columns were relabeled with ^{125}I (28) and precipitated by a double-antibody procedure (24) with 5 μl of cat or goat antiserum (15 min at 37°) followed by the addition of appropriate anti-7S globulin (18 hr at 4°). Precipitates were washed five times in 0.1 M Tris, pH 8.0/0.1 M NaCl, dissolved in 2% sodium dodecyl sulfate ($NaDodSO_4$)/0.2 M 2-mercaptoethanol, and run in 12% polyacrylamide gels containing $NaDodSO_4$ (31).

RESULTS

FeLV-Related Antigens in FeSV Pseudotypes. Nonproducer mink cell clones transformed by FeSV were radioimmunoassayed for FeLV-related antigens. No clones produced FeLV-related p30 (cf. ref. 12), gp70, or p10 (cf. ref. 32) antigens, although all expressed FeLV p15 and p12 antigens (32). To determine whether the latter antigens were packaged in FeSV-containing virions, mink and rat nonproducer cells were infected with helper viruses, and extracellular virions were radioimmune assayed. Pseudotypes produced by FeSV-transformed mink or rat cells infected with heterologous helper viruses (M7, AT-124, SSAV) contained FeLV p15 (Fig. 1*A*) and p12 (Fig. 1*B*) antigens, but neither was detected in helper viruses alone or in viral pseudotypes rescued from Kirsten sarcoma virus (KiSV)-transformed mink cells.

Infection of transformed nonproducer mink cells can lead to the expression of an endogenous mink type C virus (designated MiLV) (21). Although MiLV contains a p30 protein antigenically related to that of FeLV, it does not compete in a radioimmunoassay for FeLV p15 protein (Fig. 1*A*) and cross-reacts only minimally in the p12 assay (Fig. 1*B*). Low-level competition was seen with KiSV (M7) in the FeLV p12 assay, indicating that MiLV antigens were present (21).

Table 1. Quantitation of FeLV p15 and p12 antigens in various viral stocks

Virus	Grown in:	Assay, ng antigen/mg protein p15	p12	Helper virus p30
M7	64F2 (mink)	4,530	4,350	59,000 (M7) + 34,000 (MiLV)
M7	64F1 CL10 (mink)	13,800	11,100	49,600 (M7) + 12,400 (MiLV)
AT-124	64F1 CL10 (mink)	2,570	1,680	60,000 (MuLV) + <100 (MiLV)
AT-124	F3-NRK CL2 (rat)	4,810	3,700	44,000 (MuLV)
SSAV	F3-NRK CL2 (rat)	179	167	386,000 (SSAV)
M7	64J1 (mink)	<14	<20	28,000 (M7) + 14,000 (MiLV)
MSV (M7)	64 (mink)	<10	<7	16,000 (M7)
RD-114	64J1 (mink)	<10	<20	32,000 (RD-114) + <100 (MiLV)
MiLV	FCf2Th (dog)	<8	<16	110,000 (MiLV)
AT-124	A204 (human)	<21	<42	168,000 (MuLV)
M7	A204 (human)	<14	<28	220,000 (M7)
SSAV	NRK (rat)	<15	<30	421,000 (SSAV)
FeLV	FEC (cat)	100,000	24,000	333,000 (FeLV)

Table 1 shows the levels of FeLV p15 and p12 and helper virus p30 antigens in pseudotype viruses. FeLV-related p15 and p12 were detected only in FeSV; MSV pseudotypes, MiLV, and the various helper viruses lacked both antigens. Thus, (*i*) FeSV pseudotypes contain helper viral-independent, FeLV-related p15 and p12 antigens; (*ii*) the FeSV genome codes for both antigens, because both can be detected in viruses released from either mink or rat cells; and (*iii*) both antigens are packaged in approximately equimolar quantities.

Molecular Weights of Antigens Encoded by FeSV. Disrupted pseudotype virions were subjected to gel filtration under nondenaturing conditions, and column fractions were assayed for the presence of viral antigens. In an experiment performed with FeLV (Fig. 2*A*), discrete peaks of p30 (~ fraction 64) and p12 (~ fraction 76) were detected. Although the p15 protein aggregated (33) and was delivered in a heterodisperse region of the effluent, no significant p30, p15, or p12 activity was detected in regions corresponding to molecules larger than 70,000 daltons. Fig. 2*B* shows a similar experiment performed with an FeSV (M7) pseudotype rescued from mink cells. M7 p30 activity was found in a region corresponding to molecules of 30,000 daltons; the FeLV p15 and p12 activities were each localized to two peaks corresponding to the column void volume (fraction 31, > 220,000 daltons) and to molecules heavier than viral polymerase (~ fraction 48, 80,000–100,000 daltons). Similar results were obtained with FeSV (AT-124) obtained from rat F3-NRK CL2 cells (Fig. 2*C*), although low levels of FeLV p15 and p12 activity were also detected in regions corresponding to authentic p15 and p12 proteins.

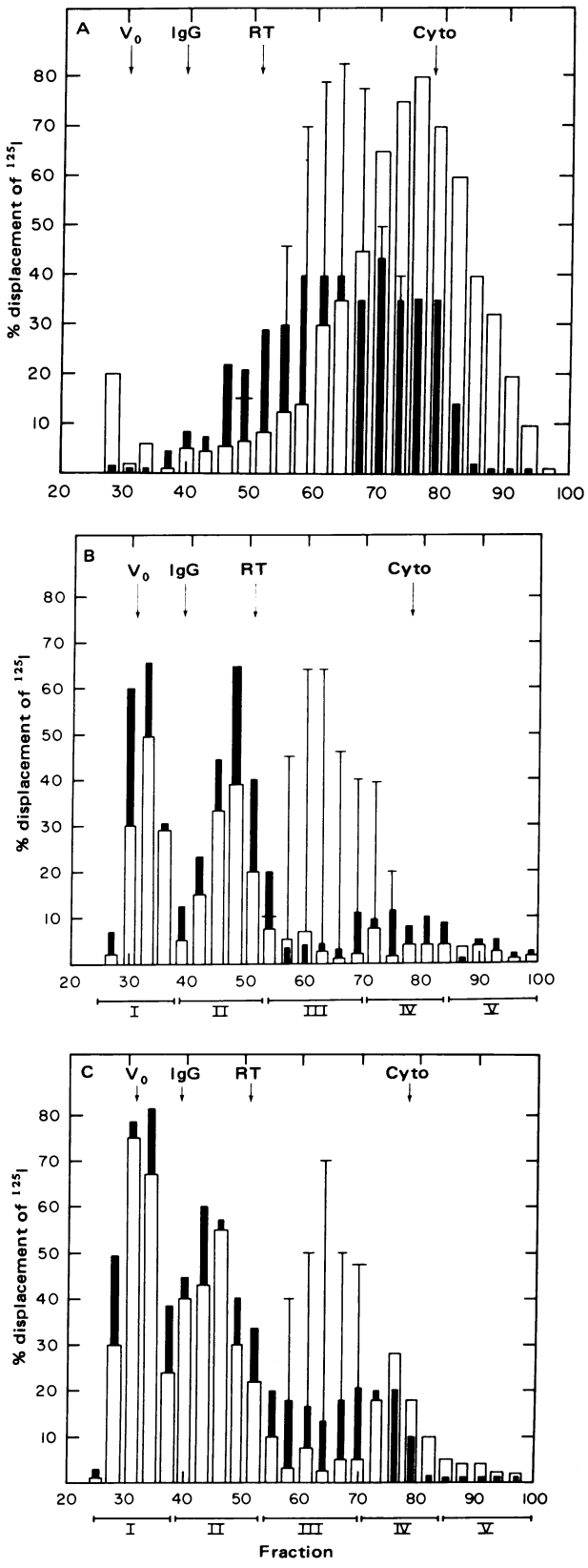

FIG. 2. Gel filtration in Sephadex G-200 of disrupted FeLV (*A*), FeSV (M28) (*B*), and FeSV (AT-124) (*C*). FeSV (M28) was obtained from mink cells; FeSV (AT-124) was from rat cells. The positions of protein markers (Cyto = cytochrome c) and reverse transcriptase (RT) are shown by arrows. FeLV p15 activity is indicated by solid bars, FeLV p12 by open bars, and helper viral p30 activity by extended lines. Fractions corresponding to those pooled for adsorptions are indicated by brackets.

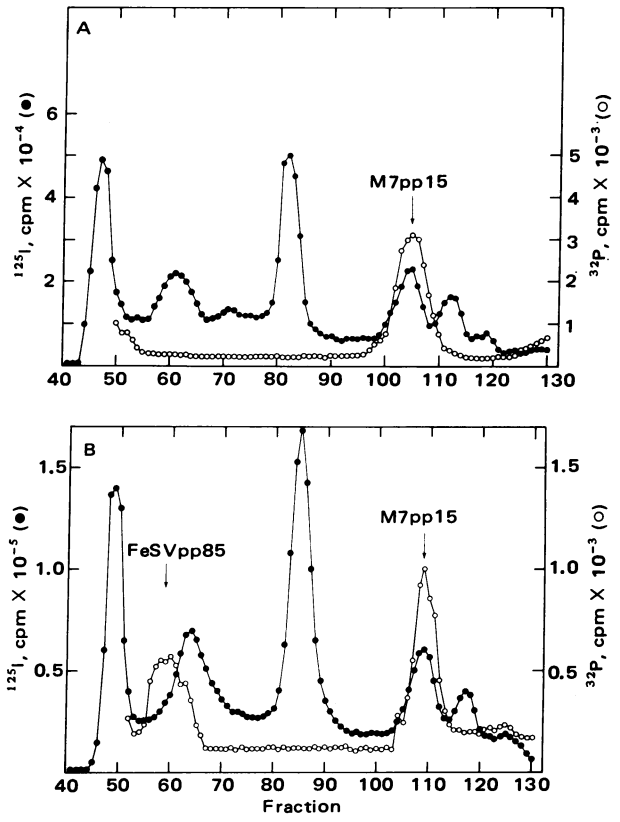

FIG. 3. Gel filtration of radiolabeled M7 (*A*) and FeSV (M7) (*B*) viruses in Bio-Gel A5M containing 6 M guanidine hydrochloride and 20 mM dithiothreitol. Arrows indicate positions of phosphoproteins. The first ^{125}I-labeled peak corresponds to the column void volume.

Because the p12 protein of FeLV is phosphorylated (34), ^{32}P-labeled FeSV pseudotypes were chromatographed under denaturing and reducing conditions to localize a ^{32}P-labeled polyprotein. Fig. 3*A* shows that, when ^{32}P- and ^{125}I-labeled baboon virus was chromatographed, six peaks of ^{125}I-labeled material were detected, corresponding to the column void volume and to the baboon viral structural proteins gp70, p30, p15, p12, and p10 (33, 35). Only the p15 protein was phosphorylated (cf. ref. 34). By contrast, two phosphoproteins were detected in labeled preparations of FeSV (M7) (Fig. 3*B*), the smaller corresponding to M7 p15 protein and the larger molecules having an apparent size of 85,000 daltons (designated pp85). All of the FeLV-related p15 and p12 activities were detected in fractions corresponding to pp85, but none of the fractions contained FeLV p30 antigen. Thus, under nondenaturing conditions (Fig. 2), two high molecular weight species of FeSV antigenic reactivity were detected but under denaturing conditions (Fig. 3) only molecules of ~85,000 daltons were seen.

Detection of FOCMA in FeSV Pseudotypes. Cat sera containing antibodies to FOCMA were adsorbed with disrupted pseudotype particles, and the adsorbed sera were tested by immunofluorescence with nonproducer, FeSV-transformed mink cells (64F3 CL7) (11). FeSV pseudotypes decreased the anti-FOCMA titer, and the degree of reduction corresponded to the quantities of FeLV p15 and p12 antigens detected by radioimmunoassay (Table 2). KiSV (M7), Moloney MSV (M7), and different helper viruses did not decrease the anti-FOCMA titer. Purified viral proteins from FeLV (including p15 and p12), RD-114, or MiLV did not affect the anti-FOCMA reactivity.

To estimate the size of FOCMA antigen in FeSV-containing

Table 2. Adsorption of anti-FOCMA serum with viruses and purified viral proteins

Adsorption sample	FeLV-related p15 or p12 antigen, μg	Serum, ml	Anti-FOCMA titer[a]
None	—	0.10	32–64
Tween/ether-disrupted virus[b]			
FeSV (AT-124)/mink	4.5	0.10	4
FeSV (AT-124)/mink	2.0	0.10	8
FeSV (M28)/mink	1.0	0.10	16
FeSV (AT-124)/rat	0.8	0.10	16–32
FeSV (AT-124)/mink	0.2	0.10	32
KiSV (M7)/mink	0.0	0.10	32
MMSV (M7)/mink	0.0	0.10	32
FeLV/cat	150	0.10	64
RD-114/human	0.0	0.10	32
M28/human	0.0	0.10	64
AT-124/cat	0.0	0.10	64
AT-124/bat	0.0	0.10	32
MiLV/dog	0.0	0.10	32
Purified viral proteins			
FeLV gp70 (40 μg)	0.0	0.05	32
FeLV p30 (100 μg)	0.0	0.05	32
FeLV p15 (100 μg)	100	0.05	32
FeLV p12 (15 μg)	15	0.05	32
FeLV dp12 (10 μg)[c]	10	0.05	32
FeLV p15 + p10 (80 μg)	40	0.05	32
RD-114 p30 (25 μg)	0.0	0.10	32
RD-114 p16 (10 μg)	0.0	0.10	32
RD-114 p12 (10 μg)	0.0	0.10	32
MiLV p30 (50 μg)	0.0	0.05	32
MiLV p15 (130 μg)	0.0	0.05	32
MiLV p12 (120 μg)	0.0	0.05	32
Fractions from Sephadex G-200[d]			
FeSV (AT-124)/mink fx I	1.0	0.05	16
FeSV (AT-124)/mink fx II	1.0	0.05	8
FeSV (AT-124)/mink fx III	0.8	0.05	16
FeSV (AT-124)/mink fx IV	0.3	0.05	32
FeSV (AT-124)/mink fx V	<0.1	0.05	64
FeSV (M28)/mink fx I	1.5	0.10	8–16
FeSV (M28)/mink fx II	1.0	0.10	16
FeSV (M28)/mink fx III	0.6	0.10	32
FeSV (M28)/mink fx IV	<0.1	0.10	64
FeSV (M28)/mink fx V	<0.1	0.10	64
Fractions from GuHCl column[e]			
FeSV (M7) fx 45–55	<0.05	0.10	64
FeSV (M7) fx 56–65	2.0	0.10	16
FeSV (M7) fx 66–80	<0.05	0.10	64
FeSV (M7) fx 81–100	<0.05	0.10	64
FeSV (M7) fx 101–127	<0.05	0.10	64
Fractions from immunoadsorbant column[f]			
Sephadex fx II	1.0	0.10	16
Unadsorbed fraction	0.0	0.05	64
Adsorbed fraction	0.9	0.05	8

Lyophilized proteins were used for adsorptions.

[a] Titers are reciprocals of highest serum dilutions giving positive immunofluorescence with 50% of the target 64F3 CL7 mink cells (11).

[b] Viral protein (1.0–1.5 mg) was used for each adsorption.

[c] The p12 protein of FeLV was dephosphorylated by using *Escherichia coli* alkaline phosphatase, with removal of 60% of covalently linked phosphate (27).

[d] Fractions correspond to pooled regions of effluents as shown by brackets in Fig. 2.

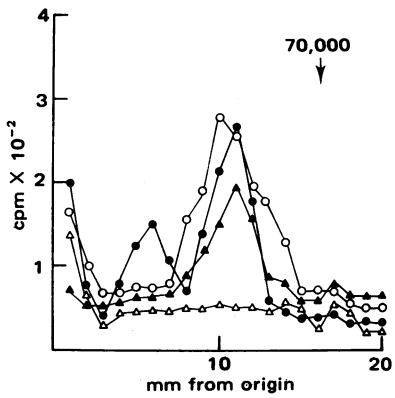

FIG. 4. Immune precipitation and $NaDodSO_4$ gel electrophoresis of fractions from guanidine hydrochloride column containing pp85. Pooled, concentrated fractions (56–65) from the column shown in Fig. 3*B* were relabeled with ^{125}I (5 μCi/μg). Labeled proteins were precipitated with various sera and run in 12% acrylamide gels. The arrow indicates the position of FeLV gp70. ●, ^{125}I-Labeled proteins prior to immune precipitation. Immune precipitates prepared with: ○, goat antiserum to disrupted FeLV; ▲, cat antiserum to FOCMA absorbed with FeLV; △, serum from a specific pathogen-free cat. No discernible protein peaks were present beyond 20 mm from the origin of the gels.

virions, fractions pooled from Sephadex G-200 (brackets, Fig. 2 *B* and *C*) as well as fractions from the guanidine hydrochloride-containing column (Fig. 3*B*) were tested for their ability to adsorb antibodies to FOCMA. Only fractions containing pp85 were effective (Table 2), suggesting that p15, p12, and FOCMA were linked in pp85. When fractions from Sephadex columns containing ~1.0 μg of p15 and p12 activity (Fig. 2, fraction II) were chromatographed on immunoadsorbants prepared with antibodies to FeLV, 90% of the p15 and p12 activities bound and were eluted with guanidine hydrochloride. Only the adsorbed fraction contained FOCMA activity (Table 2). By contrast, fractions containing FOCMA, p15, and p12 did not adsorb to immunoadsorbants prepared with purified antibodies to FeLV p30.

Fractions from guanidine hydrochloride-containing columns were also relabeled with ^{125}I and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. When fractions containing pp85 were subjected to electrophoresis, two peaks (95,000 and 85,000 daltons) were resolved (Fig. 4). The 85,000 dalton species was precipitated with goat antiserum to FeLV or with cat anti-FOCMA serum adsorbed with FeLV. By contrast, a serum obtained from a "specific pathogen-free" cat did not precipitate these molecules. Thus, FeSV codes for molecules of 85,000 daltons that are phosphorylated and contain FOCMA, p15, and p12 antigens.

DISCUSSION

Following rescue of the FeSV genome from nonproducer mink or rat cells, FOCMA and FeLV-related p15 and p12 antigens can be detected in extracellular pseudotype particles. The structural proteins p15 and p12 are packaged in equimolar amounts that correlate with the amount of FOCMA activity. Under nondenaturing and denaturing conditions, p15, p12, and FOCMA cochromatograph with phosphorylated molecules of 85,000 daltons (pp85). Molecules of this size can be precipitated

[e] Fractions were obtained from the column shown in Fig. 3*B*.

[f] FeSV (AT-124) virions were chromatographed on Sephadex G-200 and one-half of the protein in fraction II (Fig. 2) was used for serum adsorption. The remainder was applied to the immunoadsorbant, and viral protein was eluted with guanidine hydrochloride.

by both antiviral and anti-FOCMA antibodies, and FOCMA specifically binds to immunoadsorbants prepared with antibodies to FeLV. Because FeLV proteins do not themselves react with antibodies to FOCMA, we conclude that FOCMA differs from p15 and p12 but is associated with viral antigens in molecules of 85,000 molecular weight. Our data do not formally exclude the possibility, however, that more than one 85,000 molecular weight species is encoded by FeSV.

Different FeSV pseudotypes contain variable quantities of 85,000 dalton polyprotein. This may reflect variable levels of expression of FeSV sequences in different nonproducer cells or "processing" of pp85. For example, using FeSV (AT-124) we detected some processed p15 and p12 proteins, whereas in FeSV (M7) stocks, the p15 and p12 antigenic reactivities were found only in high molecular weight molecules. To date, we have not detected a ^{32}P-labeled pp85 species in stocks of FeSV (FeLV), suggesting that efficient processing might occur during rescue by a related helper virus. Recent studies (36) have indeed shown that FeSV-transformed mink cells contain molecules (p $\sim$ 90) with FOCMA, p15, and p12 activities as well as separate species containing FOCMA (p65) and the two linked FeLV polypeptides (p25).

FeLV codes for gp70, reverse transcriptase, and at least four low molecular weight proteins, p30, p15, p12, and p10 (37, 38). The latter four proteins are synthesized as a common precursor (p70) encoded by a gene (*gag*) located at the 5′ end of the viral RNA (39). The genome of FeSV shares sequences in common with those of FeLV but appears to contain additional unshared sequences (designated *sarc*) that are thought to be responsible for transformation. Mink nonproducer cells transformed by FeSV fail to express detectable quantities of FeLV-related gp70, p30, or p10, but express p15 and p12 (32). If the *gag* sequences in FeSV RNA that code for p30 and p10 were deleted, an 85,000 dalton molecule containing *gag* and FOCMA antigens might reflect translational "read through" into adjacent *pol* sequences. Alternatively, FOCMA could be a *sarc* gene product that functions as a transforming protein.

Although FOCMA is not detected on FeLV-infected fibroblasts, it is found on FeLV-induced neoplastic lymphoid cells (2, 40). If FOCMA were only encoded by FeSV, FeLV would have to activate endogenous sequences coding for FOCMA in cat lymphoid cells. By contrast, FOCMA could contain a set of antigenic determinants found in an unprocessed or otherwise modified FeLV polyprotein that accumulates only in transformed lymphoid tissues.

If FOCMA detected in pseudotype virions is functionally analogous to that found on transformed cells, animals immunized with inactivated pseudotypes should be protected against leukemias and sarcomas induced by FeLV and FeSV. The localization of FOCMA in virions also affords a relatively simple approach to purifying the antigen and determining its efficacy in the treatment of a natural, viral-induced, neoplastic disease.

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1. Essex, M. (1977) *Contemp. Top. Immunobiol.* **6**, 71–106.
2. Hardy, W. D., Jr., Zuckerman, E. E., MacEwen, E. G., Hayes, A. A. & Essex, M. (1977) *Nature*, **270**, 249–251.
3. Essex, M., Stephenson, J. R., Hardy, W. D., Jr., Cotter, S. M. & Aaronson, S. A. (1977) *Cold Spring Harbor Conference on Cell Proliferation*, eds. Watson, J. D., Hiatt, H. H. & Winsten, J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Vol. 4, 1197–1214.
4. Essex, M., Klein, G., Snyder, S. & Harrold, J. B. (1971) *Nature* **233**, 195–196.
5. Essex, M., Sliski, A., Hardy, W. D., Jr. & Cotter, S. M. (1976) *Cancer Res.* **36**, 640–645.
6. Essex, M., Jakowski, R. M., Hardy, W. D., Jr., Cotter, S. M., Hess, P. & Sliski, A. (1975) *J. Natl. Cancer Inst.* **54**, 637–641.
7. Jarrett, W., Jarrett, O., Mackey, L., Laird, H., Hood, C. & Hay, D. (1975) *Int. J. Cancer* **16**, 134–141.
8. Olsen, R. G., Hoover, E. A., Mathes, L. E., Heding, L. & Schaller, J. P. (1976) *Cancer Res.* **36**, 3642–3649.
9. Essex, M., de Noronha, F., Oroszlan, S. & Hardy, W. D., Jr. (1977) *Proceedings of the Third International Symposium on Detection and Prevention of Cancer*, ed. Nieburgs, H. (Marcel Dekker Inc., New York), Vol. 2, in press.
10. Stephenson, J. R., Essex, M., Hino, S., Aaronson, S. A. & Hardy, W. D., Jr. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1219–1223.
11. Sliski, A., Essex, M., Meyer, C. & Todaro, G. J. (1977) *Science* **196**, 1336–1339.
12. Henderson, I. C., Lieber, M. M. & Todaro, G. J. (1974) *Virology* **60**, 282–287.
13. Huebner, R. J., Hartley, J. & Rowe, W. (1966) *Proc. Natl. Acad. Sci. USA* **56**, 1164–1169.
14. Giard, D. J., Aaronson, S. A., Todaro, G. J., Arnstein, P., Kersey, J. H., Dosik, H. & Parks, W. P. (1973) *J. Natl. Cancer Inst.* **51**, 1417–1423.
15. Duc-Nguyen, H., Rosenblum, E. N. & Ziegel, R. F. (1966) *J. Bacteriol.* **92**, 1133–1140.
16. Benveniste, R. E., Lieber, M. M., Livingston, D. M., Sherr, C. J., Todaro, G. J. & Kalter, S. S. (1974) *Nature* **248**, 17–20.
17. Todaro, G. J., Sherr, C. J., Benveniste, R. E., Lieber, M. M. & Melnick, J. L. (1974) *Cell* **2**, 55–61.
18. Theilen, G. H., Gould, D., Fowler, M. & Dungworth, D. L. (1971) *J. Natl. Cancer Inst.* **47**, 881–889.
19. Todaro, G. J., Arnstein, P., Parks, W. P., Lennette, E. H. & Huebner, R. J. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 859–862.
20. McAllister, R. M., Nicolson, M., Gardner, M. B., Rongey, R. W., Rasheed, S., Sarma, P. S., Huebner, R. J., Hatanaka, M., Oroszlan, S., Gilden, R. V., Kabigting, A. & Vernon, L. (1972) *Nature New Biol.* **235**, 3–6.
21. Sherr, C. J., Benveniste, R. E. & Todaro, G. J. (1978) *J. Virol.*, in press.
22. Rickard, C. G., Post, J. E., de Noronha, F. & Barr, L. M. (1969) *J. Natl. Cancer Inst.* **42**, 987–1014.
23. Sherr, C. J. & Todaro, G. J. (1974) *Virology* **61**, 168–181.
24. Sherr, C. J., Fedele, L. A., Benveniste, R. E. & Todaro, G. J. (1975) *J. Virol.* **15**, 1440–1448.
25. Sen, A., Sherr, C. J. & Todaro, G. J. (1976) *Cell* **7**, 21–32.
26. Scolnick, E. M., Parks, W. P. & Livingston, D. M. (1972) *J. Immunol.* **109**, 570–577.
27. Sen, A., Sherr, C. J. & Todaro, G. J. (1977) *Cell* **10**, 489–496.
28. Greenwood, F. C., Hunter, W. M. & Glover, J. S. (1963) *Biochem. J.* **89**, 114–123.
29. Cuatrecasas, P. & Anfinsen, C. B. (1971) in *Methods in Enzymology*, ed. Jakoby, W. B. (Academic, New York), Vol. 22A, pp. 345–369.
30. Essex, M. & Snyder, S. P. (1973) *J. Natl. Cancer Inst.* **51**, 1007–1012.
31. Laemmli, U. K. (1970) *Nature* **227**, 680–685.
32. Khan, A. S. & Stephenson, J. R. (1977) *J. Virol.* **23**, 599–607.
33. Barbacid, M., Stephenson, J. R. & Aaronson, S. A. (1977) *Cell* **10**, 641–648.
34. Pal, B. K., McAllister, R. M., Gardner, M. B. & Roy-Burman, P. (1975) *J. Virol.* **16**, 123–131.
35. Sen, A., Sherr, C. J. & Todaro, G. J. (1978) *Virology*, **84**, 99–107.
36. Stephenson, J. R., Khan, A. S., Sliski, A. H. & Essex, M. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5608–5612.
37. Nowinski, R. C., Fleissner, E., Sarkar, N. H. & Aoki, T. (1972) *J. Virol.* **9**, 359–366.
38. Graves, D. C. & Velicer, L. F. (1974) *J. Virol.* **14**, 349–365.
39. Okasinski, G. F. & Velicer, L. F. (1977) *J. Virol.* **22**, 74–85.
40. Essex, M., Sliski, A., Hardy, W. D., Jr., de Noronha, F. & Cotter, S. M. (1977) in *Comp. Leukemia Research 1977*, eds. Bentvelzen, P. & Yohn, D. S. (Elsevier, North Holland, Amsterdam), in press.