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Isolation from Cats of an Endogenous Type C Virus with a Novel Envelope Glycoprotein

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A search for variant endogenous cat viruses led to a novel isolate. Although the major envelope glycoprotein of this virus was similar in size to that of an RD-114-like virus that was coisolated, it was unrelated to RD-114 or feline leukemia virus by immunological and biological criteria. This degree of dissimilarity suggests a different evolutionary progenitor from that for the RD-114 and feline leukemia virus viral envelopes. The novel virus did, however, code for *gag* gene polypeptides which are closely related to RD-114 virus. Neither the novel isolate nor the RD-114-like coisolate induced foci in S^+L^- cat cells which restrict focus induction by RD-114 virus. This suggests that the two viruses share a common genomic target of restriction which resides outside of the *env* region.

Previous studies have reported that the replication of RD-114-like endogenous cat virus is restricted intracellularly by feline embryo fibroblast (FEF) cells (11–14). More recent studies in this laboratory have shown that this restriction is not absolute and that all FEF cells tested, including one embryo pool previously reported to restrict RD-114 virus replication (14), do in fact permit virus replication (D. Haapala, manuscript in preparation). We can, however, differentiate two types of FEF cells based on whether they are transformed by the RD-114 pseudotype of Moloney murine sarcoma virus (MSV). We have found that cells derived from permissive embryos are as sensitive as mink lung cells to transformation by MSV(RD-114), whereas restrictive FEF cells do not transform upon infection with MSV-(RD-114) unless coinfected with a nonrestricted helper virus (D. Haapala, manuscript in preparation). We have also shown that S^+L^- cells (3, 5) derived from the two cell types give results which are consistent with this observation. Although each serves as an efficient indicator for feline leukemia virus (FeLV) and nonfeline viruses (including murine mink cell focus-forming virus and primate type C viruses), RD-114 causes focus formation in the permissive S^+L^- cell line but not in the S^+L^- cells made from the restrictive phenotypes (D. Haapala, manuscript in preparation).

These results suggested to us that variants of the endogenous cat virus might exist which have a tropism for each of the two FEF phenotypes. This reasoning was partly based on studies of the intracellular restriction of murine leukemia viruses, which have served to define two mouse genotypes and two corresponding virus genotypes at the *Fv-1* locus (20, 35; discussed in reference 23). The two allelic forms, *Fv-1n* and *Fv-1b*, are typified, respectively, by the NIH/Swiss and BALB/c inbred mouse strains. Similarly, murine leukemia viruses are described as N-tropic or B-tropic based on their relative ability to infect cells derived from the two genotypes. Although neither virus is completely excluded by either cell type in mass infections (7, 9, 35), N-tropic virus infection of *Fv-1b* cells (or B-tropic virus infection of *Fv-1n* cells) requires two virus particles. The primary infection renders the cell temporarily permissive for infection by the same virus type, which results in a two-hit infectivity curve (9).

Since documented variants of RD-114 do not exist, we began our search for host range variants in virus stocks derived from various cell lines. We included the MSV(CCC) virus 6DDK in this search since it was reported to have a ratio of MSV to its endogenous helper virus of 100:1 (33) compared with the more usual ratios of 4:1 to 1:4 (5, 38). As discussed in this paper, we did isolate a novel helper virus from this virus stock, although not of the type expected. We now report the characterization of a virus having the same host range and major core proteins as RD-114 virus but having a surface glycoprotein immunologically distinct from RD-114 and the three known FeLV subtypes.

MATERIALS AND METHODS

Cell culture and virus production. All cells were grown in McCoy 5A medium supplemented with 15% heat-inactivated fetal calf serum and PSN antibiotics (GIBCO Laboratories). Virus stocks were prepared from rapidly growing cells in roller bottles. The supernatant fluid was harvested after 18 h, clarified at 2,000 × *g* for 15 min, filtered through a Nalgene filter (pore size, 0.45 μm), and then sampled and stored at −70°C until use.

Helper viruses were assayed on mink S^+L^- (33) or PG-4 S^+L^- cat cells. Moloney MSV was assayed on cat clone G355-5 with or without the addition of optimal helper virus (11).

The general method for virus infection involved plating 2 × 10^5 cells in a 60-mm petri dish. The next day, the cells were treated with 1 ml of DEAE-dextran (25 μg/ml) for 30 min and then washed two times with 1 ml of growth medium. The virus, in 0.2 ml of medium, was then added to the cells and allowed to adsorb at 37°C with periodic agitation. After 60 min, 4 ml of fresh medium was added to each dish. The cell media were changed after days 3 and 5, and foci were counted at day 6 or 7.

Isolation of helper viruses by the terminal-focus method. Virus stocks from 6DDK cells producing M-1 Moloney MSV(CCC) (38) were plated at limiting dilutions on G355-5 cells after sonication for 10 s at 100 W of output in an ice bath. This treatment was sufficient to disrupt virus aggregates (unpublished data). Individual foci of transformed cells

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were recovered by trypsinization with the aid of cloning cylinders, and these cells were transferred to individual flasks from which virus stocks were prepared for further characterization.

Virus neutralization. The immunological properties of virion envelopes were analyzed by using hyperimmune sera. Virus stocks (90 μl) were incubated with 10 μl of each antiserum dilution at room temperature for 120 min. The viruses were then diluted and plated on S^+L^- indicator cells as described above. Controls with normal goat serum were included in each study.

Virus interference studies. Envelopes of the virus isolates were characterized by using interference as a criterion as described by Sarma and Log (39). All pseudotypes of MSV were prepared by rescue of MSV from PG-4 S^+L^- cells since these cells do not release detectable helper virus spontaneously or after chemical induction (unpublished data). Interference was measured in clone G355-5, which is the parent to the PG-4 S^+L^- line. FeLV subtypes A (Sarma), B (Snyder-Theilen), and C (Sarma) were kindly provided by Padman Sarma, National Cancer Institute.

Characterization of viral proteins. Actively growing cultures (ca. 4×10^6 cells per 75-cm^2 flask) were labeled for 16 h with [^{14}C]glucosamine (54.2 Ci/mmol; New England Nuclear Corp.) at a concentration of 2.5 μCi/ml. Supernatant fluids were clarified at 10,000 × *g* for 10 min. Virions were purified by sedimentation through 30% glycerol–NTE (NTE is 0.1 M NaCl, 10 mM Tris hydrochloride [pH 7.4], 1 mM EDTA), onto a 70% glycerol–NTE cushion. The virions were then diluted fivefold and pelleted by centrifugation (105,000 × *g*, 60 min).

Samples of virus representing equal volumes of supernatant fluid were solubilized in either lysis buffer or electrophoresis sample preparation buffer (24, 38, 40). Virions in lysis buffer were treated with a Dounce homogenizer and clarified at 10,000 × *g* for 20 min. The supernatant fluids were then incubated with normal goat serum for 1 h at 37°C after which 50 μl of a 10% suspension of formaldehyde-fixed *Staphylococcus aureus* was added (37). After incubation for 1 h at 37°C, the samples were clarified at 6,000 × *g* for 10 min and treated with 10 μl of hyperimmune goat serum against RD-114 gp70 (pool 79s-738). After 16 h at 4°C, the immune complexes were precipitated by adding *S. aureus* suspension as described above, and the pellets were washed three times in lysis buffer. The immunoprecipitated polypeptides were solubilized in electrophoresis sample buffer at 95°C for 3 min. The samples were clarified by centrifugation (6,000 × *g*, 10 min), and the supernatants were analyzed on 10% polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS) (38) and treated with En^{3}Hance (New England Nuclear Corp.) before autoradiography with Kodak SB film.

Viral proteins other than glycoproteins were characterized by electroblotting and immunoautoradiography (41) as described by Morgan et al. (27). Briefly, viral proteins of L1LL and L2LL were separated by SDS-polyacrylamide gel electrophoresis and then transferred to activated diazobenzyloxymethyl paper (Schleicher & Schuell, Inc.). Replicate strips of protein-bound paper were reacted with various antisera overnight followed by treatment with ^{125}I-labeled protein A. The paper strips were then thoroughly washed and used for autoradiography.

RESULTS

Isolation of active helper virus in cat cells. All 6DDK virus stocks, unlike other pseudotypes of Moloney MSV we have tested, had a large excess of MSV over the associated helper virus. We therefore considered these a possible source of variant endogenous helper virus. We attempted to isolate this virus by diluting the virus stock after sonication to disrupt virus aggregates and then by isolating foci resulting from MSV plus the endogenous helper virus. A virus stock prepared from the third terminal focus obtained in this manner was found to contain 2×10^6 focus-inducing units in PG-4 cells and 3×10^3 focus-forming units of MSV. This virus was plated at limiting dilutions to obtain a helper virus stock free of MSV. We found that, upon passage, four of nine plates infected with 0.2 ml of a 10^{-6} dilution produced focus-inducing virus on PG-4 cells. Of these plates, one produced virions which gave foci of two distinct types on PG-4 cells. We found that this virus stock was not neutralized by anti-FeLV serum and, surprisingly, that focus-inducing virus also remained after treatment with anti-RD-114 serum. The resulting large and small focus types were picked from a limiting dilution with cloning cylinders, and virus stocks were prepared at passage 1. The results of these studies with the two foci, LD-1 and LD-2, are shown in Table 1. Passage 1 LD-1 virus treated with anti-RD-114 plus anti-FeLV sera was completely neutralized, whereas LD-2 virus was not. Host range studies of these viruses are also shown in Table 1. Both viruses produced foci on the permissive cat cell line PG-4 and mink F648-1 S^+L^- cells as expected (33), but neither produced foci in clone 819 S^+L^- cat cells, which are restrictive for RD-114-like viruses (14).

To purify the two virus types, the LD-1 stock was plated on clone G355-5 cells to obtain a limiting dilution, and the LD-2 stock was again treated with antisera against FeLV and RD-114 and plated on PG-4 cells. A virus stock obtained from this PG-4 terminal focus was simultaneously plated on clone G355-5 and on PG-4 cells, and the endpoint on PG-4 cells was used to estimate a limiting dilution on clone G355-5 cells for LD-2 production. The resulting two stocks, called, respectively, L1L and L2L, were examined further.

Foci formed by the small focus variant L2L after treatment of the virus stock with normal goat serum are shown in Fig. 1A, and those formed after treatment with anti-RD-114 gp70 plus anti-FeLV are shown in Fig. 1B. The larger foci induced by virus from RD-114 cells are shown in Fig. 1C. This RD-114 stock was completely neutralized by treatment with anti-RD-114 gp70 but not by anti-FeLV serum. Also shown for comparison are the much larger foci induced by FeLV (Fig. 1D).

TABLE 1. Characterization of viruses obtained from limiting-dilution foci

Virus passage[a]	Treatment[b]	No. of foci produced on indicator S^+L^- cell line		
		PG-4	819	F648-1
LD-1				
P_0		3.2×10^5	0	1.2×10^5
P_1[a]	NGS	1.5×10^5		
P_1	RF	0		
LD-2				
P_0		1.2×10^3	0	2.2×10^3
P_1	NGS	1.0×10^4		
P_1	RF	2.5×10^3		

[a] P_0, Before passage; P_1, passage 1.

[b] Passage 1 harvests from both LD-1 and LD-2 foci were treated with normal goat serum (NGS) or a 1:20 dilution of anti-RD-114 plus anti-FeLV sera (RF) before plating on PG-4 cells.

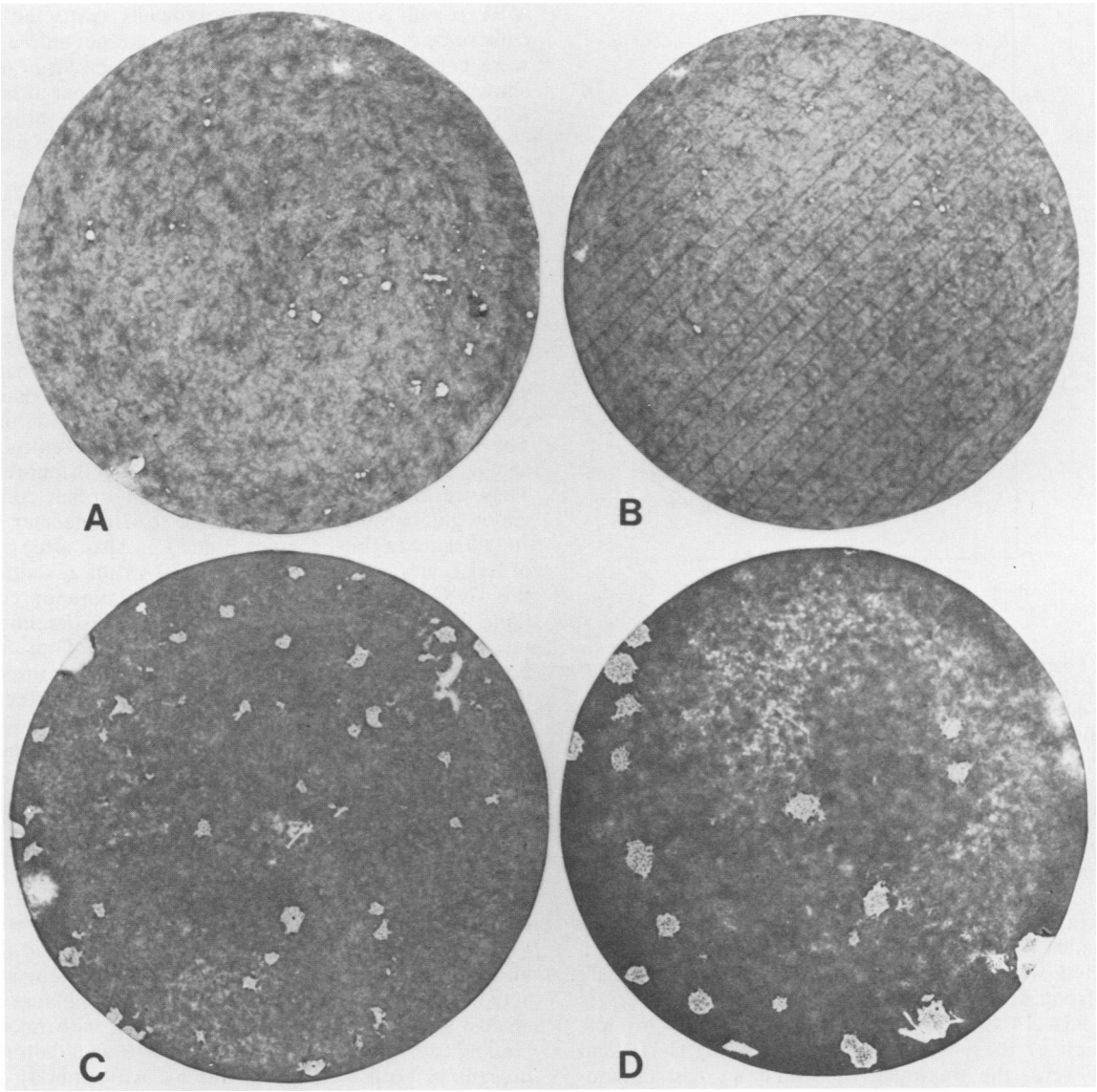

FIG. 1. Foci appearing on PG-4 cells after May-Grunwald-Giemsa staining. The cells had been infected 7 days previously and handled as described in the text. Panel A, the appearance of two distinct types of foci after infection with L2L, P_1 treated with normal goat serum (see Table 1); panel B, the same virus stock after treatment with anti-RD-115 plus anti-FeLV sera; panels C and D, the type of foci induced by RD-114 (C) and FeLV (D), without antiserum treatment.

The L1L and L2L stocks were again plated on clone G355-5 cells at limiting dilutions. The resulting virus-producing cells were propagated in roller bottles from which overnight harvests of viruses, called L1LL and L2LL, were obtained for biological and immunological characterization.

Biological characterization of the L1LL and L2LL coisolates. The rigorously purified stocks of the large and small focus variants were tested for envelope specificity by neutralization. The results of this study with goat anti-RD-114 gp70 serum (Fig. 2) indicate that L1LL shares the coat antigenicity of RD-114 but that the L2LL isolate does not.

Another sensitive measure of virus envelope specificity for cell receptors, viral interference, has been used by Sarma and Log (39) to differentiate three subtypes of FeLVs. We therefore prepared the L1LL and L2LL pseudotypes of Moloney MSV by infecting PG-4 S^+L^- cells. Individual foci induced by each virus were picked with cloning cylinders and subcultured to obtain stocks of Moloney MSV pseudotyped by each helper. These stocks were then used to infect G355-5 control cells as well as G355-5 cells chronically producing L1LL, L2LL, or the A, B, or C subtypes of FeLV. The various G355-5 cells were infected with twofold dilutions of each pseudotype per se without the addition of exogenous helper virus. This study, summarized in Table 2, showed a lack of viral interference between the L1LL and L2LL pseudotypes. Additionally, MSV(L1LL) and MSV(L2LL) both produced foci in the cell lines infected with each of the three FeLV subtypes. We conclude that L2LL is therefore not a known FeLV subtype by the criterion of viral interference.

Questions concerning the origin and frequency of occurrence of L2LL-related viruses were next addressed. RD-114-like viruses from different sources and with differing passage histories were included. The history of the 6L and 6DDK cells is discussed above. The 8L cells have a related history in that the virus used to inoculate the restrictive FEF cells

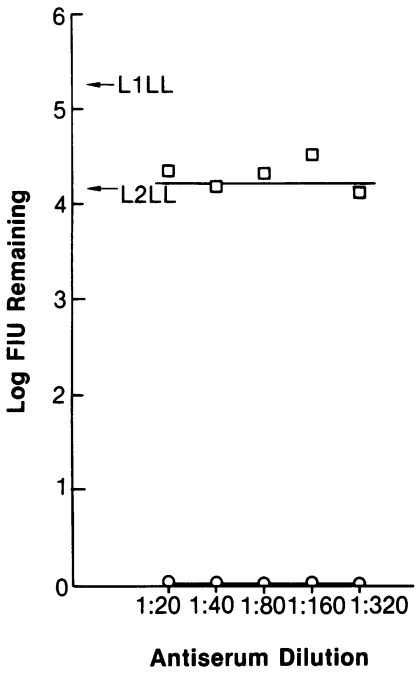

FIG. 2. Focus-inducing activity of the rigorously purified virus coisolates L1LL and L2LL after treatment with anti-RD-114 gp70 at increasing dilutions. The arrows indicate the titer of each virus obtained after treatment with a 1:20 dilution of normal goat control serum.

was the MSV(CCC) virus spontaneously released from a producing clone of 819 cells. This virus was passaged in FEF cells in limiting dilution to separate the endogenous helper from the associated MSV. BK572 is an embryonic cat cell from the Naval Biological Laboratory which we found to be a spontaneous virus producer. CV-9 is a restrictive FEF cell line infected with terminally diluted virus spontaneously released from a CRFK-derived (6) CCC clone. K228 is authentic RD-114 virus produced by RD-114 cells (26). We treated each of these virus stocks with anti-RD-114 and anti-FeLV sera, the treatment by which we first demonstrated the L2LL variant. However, no stock examined except the 6L stock from which L2LL was purified contained detectable envelope variants (Table 3). This was also true of the 6DDK stock from which the endogenous 6L helper virus was obtained by passage in G355-5 cells. It is therefore possible that L2LL originated in the G355-5 cell line rather than the 6DDK stock. However, it is likely that L1LL and L2LL were phenotypically mixed in the 6L stock since the L1LL-producing focus was obtained after treatment of this virus stock with anti-RD-114 serum. The L2LL virus could therefore be present as the L1LL pseudotype in some of the virus stocks examined and not be detected after the neutralization method used.

To further characterize the envelopes variant L2LL, the host range of this virus was compared with those of L1LL and RD-114 viruses. Neither L1LL nor L2LL induced foci in the restrictive cat S^+L^- cell M10-14-3 or in mouse D-56 cells, whereas both viruses induced foci in permissive PG-4 and mink F648-1 cells (Table 4). We confirmed this lack of focus induction in M10-14-3 cells by use of an infectious-center assay (4). Neither L1LL- nor L2LL-producing cells caused foci when plated on the restrictive S^+L^- cell line (<1 per 30,000 cells [data not shown]). We also found that both MSV pseudotypes were phenotypically restricted by FEF cells since neither pseudotype produced foci unless the cells were coinfected with nonrestricted helper virus (data not shown). Taken together, these data show that although the L2LL virus isolate has a novel envelope, it is subject to the same intracellular virus restriction mechanism of cat cells as the RD-114-like virus isolate L1LL.

Biochemical characterization of the L1LL and L2LL coisolates. The above experiments have demonstrated that the L2LL virus isolate codes for an envelope biologically different from those of the known cat viruses. To obtain information about the chemical nature of this L2LL protein, we grew L1LL and L2LL virus-producing cells in the presence of [^{14}C]glucosamine and purified the supernatant virus by polyethylene glycol precipitation and banding in sucrose by standard methods (32). Both viruses banded at a peak density of 1.16 g cm^{-3}, typical for type C viruses (29). The proteins from each banded virus were extracted and analyzed by SDS-polyacrylamide gel electrophoresis (38). These results (Fig. 3) showed that both viruses contained a major glycoprotein corresponding to a molecular mass of 70,000 daltons (Fig. 3, lanes B and C). Also, after treatment of L1LL virus with anti-RD-114 gp70 serum, essentially only this 70,000-dalton glycoprotein was immunoprecipitated (lane E). The gp70 of L2LL virus was not immunoprecipitated under identical conditions (Fig. 3F). Thus, although both viruses contained a major glycoprotein corresponding in size to the RD-114 envelope protein, immunoprecipitation data, like the biological data presented above, show that the L2LL and the L1LL (RD-114-like) coisolates did not cross-react.

Other proteins composing the virions of the coisolates were next studied. Proteins from disrupted particles were electrophoresed and electrotransferred onto diazobenzyloxymethyl paper. Replicates of viral proteins were exposed to five different antisera directed against the internal core proteins or the external envelope protein of various type C viruses. These data (Fig. 4) demonstrated that both viruses contained a ~28,000-dalton core protein which reacted with anti-RD-114 serum (serum 1). The sera directed against FeLV p10, another core protein with nucleic acid binding capacity (27), also bound to a 10,000-dalton protein present in both viruses (serum 5). An antibody directed against a core protein with a similar function from bovine leukemia virus (27) (serum 4) did not bind to proteins of either L1LL or L2LL.

Contrasting with these results were those obtained with two antisera directed against p15-p20 envelope-associated antigens. One of these sera, sp-9, directed against a synthetic peptide based on a Moloney leukemia virus sequence (T. Copeland and S. Oroszlan, manuscript in preparation) is antiserum 2 in Fig. 4. This serum reacts with a p20 of L1LL

TABLE 2. Viral interference studies

Indicator cell (type of virus produced)	No. of foci in virus pseudotype:[a]	
	MSV(L1LL)	MSV(L2LL)
G-5	3.5	2.5
G-5(L1LL)	<0.7[b]	1.9
G-5(L2LL)	3.1	<0.7[b]
G-5(FeLV A)	3.1	2.5
G-5(FeLV B)	3.3	2.3
G-5(FeLV C)	3.2	2.7

[a] Log_{10} focus-forming virus per milliliter.
[b] Less than one focus per 0.2 ml of the undiluted virus stock.

TABLE 3. Occurrence of non-RD-114, non-FeLV in endogenous virus-producing cells

Antiserum treatment[a]	Titer[b] of virus obtained from cell line:					
	6L	6DDK	8L	BK572	CV-2	K228
NGS	5.5×10^6	6.0×10^4	1.2×10^6	6.2×10^4	1.6×10^5	9.5×10^6
1:20 RD	1.8×10^3	0	0	0	0	0
1:20 FeLV	2.8×10^6	6.0×10^4	ND[c]	2.8×10^4	1.0×10^5	ND
1:20 RAbs	4.5×10^2	0	0	0	ND	0
1:80 RAbs	2.0×10^3	ND	ND	ND	ND	0

[a] NGS, Normal goat serum; RD, anti-RD-114 gp70; FeLV, anti-FeLV Rickard serum; RAbs, anti-RD-114 gp70 serum absorbed twice with packed clone G355-5 cells.
[b] Focus-inducing units per milliliter of virus detected in PG-4 cells after treatment with antiserum.
[c] ND, Not done.

but not with proteins contained in L2LL. The other anti-p20^{env} serum was prepared against purified transmembrane proteins of replication-competent avian reticuloendotheliosis virus (15). This is also a broadly reacting serum with activity against all type C and type D viruses studied (W. P. Tsai and S. Oroszlan, manuscript in preparation). This serum (Fig. 4, serum 3) also reacted strongly with the RD-114-like L1LL virus, but no reaction occurred with L2LL virion proteins. We conclude from these data that both viruses share antigens with RD-114 virus, including the major core antigen p28 and the nucleic acid-binding polypeptide p10. However, both the gp70 and p15-p20^{env} polypeptides of L2LL lack cross-reactivity with RD-114 and the L1LL coisolate.

DISCUSSION

Domestic cats and their associated type C retroviruses received major attention in the last decade. The fact that both FeLV and RD-114 viruses infect human cells (13, 26, 28) was of particular concern because of the intimate association of these species and particularly since other well-studied mammalian type C viruses such as ecotropic (25) Rauscher and Moloney murine leukemia viruses and mouse mammary tumor virus did not infect human cells. Later observations on the horizontal spread of FeLV-induced leukemia in cats (19, 22) have served as an important model for the study of the control of a virulent form of an endogenous virus (reviewed in reference 18). More recently, another sequela of FeLV-induced disease, i.e., immunosuppression in its natural host (1, 34), has helped focus attention on the possible role of retroviruses in the etiology of human acquired immune deficiency syndrome (2, 10, 16; Montagnier et al. [*in* R. C. Gallo, M. E. Essex, and L. Gross, ed., *Human T-cell Leukemia and Lymphoma Viruses*, in press]). As mentioned above, the studies reported here arose from yet another important feature of the cat virus system, namely, the intracellular control of endogenous RD-114-like viruses. Although we were unable to isolate an endogenous virus immune to the intracellular control of restrictive FEF cells, we did find a previously undetected virus with a novel envelope glycoprotein. Four types of evidence support the conclusion that the L2LL isolate has an envelope unrelated to either RD-114 or the known FeLVs. These include the lack of (i) neutralization by specific antisera, (ii) viral interference, (iii) immunoprecipitation of glucosamine-labeled gp70 from purified L2LL virus by anti-RD-114 gp70, and (iv) L2LL cross-reactions with two broadly reactive anti-p15-p20^{env} sera which cross-react with L1LL. These data indicate a degree of genetic dissimilarity between the L1LL and L2LL isolates as great as that between the RD-114 virus and the FeLVs or between the murine viruses and FeLV (17, 30) and indicate that the L2LL envelope derives from a different evolutionary progenitor. That L2LL-related viruses have previously escaped detection (36) indicates that they are expressed only rarely although, as our data show, expression can lead to a functional virus. The reason for this lack of expression poses an interesting subject for further studies of the L2LL virus. Ongoing studies, including restriction endonuclease maps of linear proviral DNAs together with additional polypeptide studies, indicate that the L2LL virus may be an envelope gene recombinant between an RD-114-like virus and the L2LL-specific sequence which is present in the domestic cat genome (D. Haapala, K. Denniston, and W. Robey, manuscript in preparation).

TABLE 4. Host range studies of the L1LL and L2LL coisolates and RD-114 virus

Virus isolate	No. of focus-inducing viruses[a] on S^+L^- indicator cell line:			
	PG-4	M10-14-3	F648-1	D56
L1LL	1.9×10^6	0	1.0×10^6	0
L2LL	5.0×10^5	0	3.5×10^5	0
RD-114	1.3×10^6	0	2.0×10^6	0

[a] Per milliliter of culture fluid.

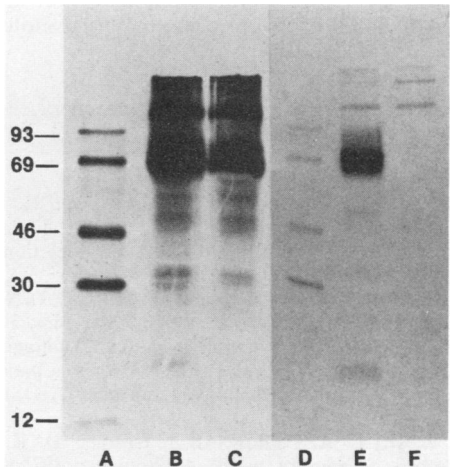

FIG. 3. Autoradiograph of proteins metabolically labeled with [^{14}C]glucosamine and separated by SDS-polyacrylamide gel electrophoresis. Lane B, L1LL virion proteins; lane C, L2LL virion proteins; lanes E and F, L1LL and L2LL proteins, respectively, after immunoprecipitation with anti-RD-114 gp70 serum. Molecular size standards (lanes A and D) included phosphorylase B, bovine albumin, ovalbumin, carbonic anhydrase, and cytochrome *c*.

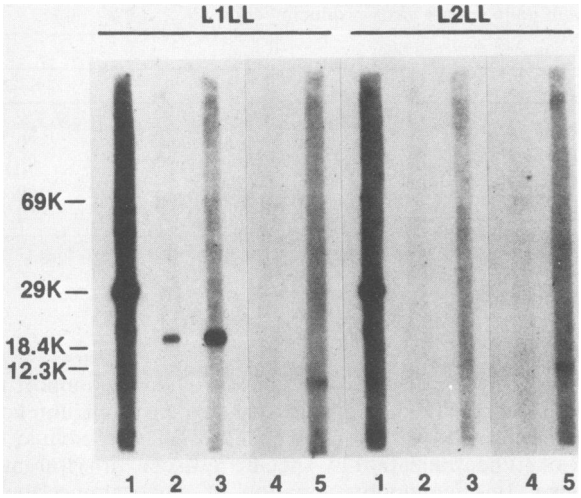

FIG. 4. Autoradiograph of L1LL and L2LL proteins reacted with five antisera directed against different viral proteins. Sera (lanes): 1, anti-RD-114; 2, sp-9 $p15^{env}$; 3, reticuloendotheliosis virus $p20^{env}$; 4, bovine leukemia virus p10; and 5, FeLV p10. Replicate electroblotted viral proteins were reacted with a 1:200 dilution of each antiserum followed by treatment with ^{125}I-labeled protein A.

Data presented in this paper leave unanswered the question of whether the intracellular restriction of endogenous RD-114-like virus is a genetic trait with more than one allele. However, our data may have a direct bearing on the nature of this restriction. We have shown that although the envelopes of the L1LL and L2LL coisolates are unrelated by four criteria, both viruses appear subject to the same restriction mechanism as evidenced by their inability to induce foci in the M10-14-3 cell line. It has not been clearly established that the target of the *Fv-1* restriction mechanism in mice is determined by the viral p30 *gag* gene (8, 21, 31). Since the *gag* genes of L1LL and L2LL appear to be identical, the restriction of endogenous viruses by randomly bred domestic cats could also be directed against a viral *gag* gene target. If this proves to be the case, the intracellular restriction of cat viruses will serve to generalize this model for the control by a mammalian cell of its endogenous virus sequences.

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