Interference with Superinfection and with Cell Killing and Determination of Host Range and Growth Kinetics Mediated by Feline Leukemia Virus Surface Glycoproteins

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The functions of the surface glycoproteins (SU) of feline leukemia viruses (FeLVs) are of interest since these proteins mediate virus infection and interference and are critical determinants of disease specificity. In this study, we examined the biochemical and genetic determinants of SU important to virus entry and cell killing. In particular, we developed and used vesicular stomatitis virus (VSV)/FeLV pseudotype virus interference assays to determine interference subgroupings and assess mechanisms of host cell restriction. We also assessed roles of SU in virus growth kinetics and in the inhibition of cell killing caused by superinfection with cytopathic virus. Subgroup classification by VSV/FeLV pseudotype assay was in agreement with that defined previously by focus interference assay and was found to be determined by changes near the N terminus of SU for FeLV subgroups A (FeLV-A) and C. Virus host range restriction was found to be mediated at the level of virus entry in most cases, although postentry events mediated restriction in the failure of a subgroup A-like, T-cell cytopathic and immunodeficiency-inducing clone (FeLV-FAIDS-EECC) to replicate in feline fibroblasts. FeLV-FAIDS-EECC-induced cell killing was also inhibited by prior infection with one of two FeLV-A isolates. This inhibition could be conveyed by as few as four amino acid changes near the N terminus of the FeLV-A SU and also appeared to be mediated at a postentry level. Lastly, the SU-coding sequence was also found to determine differences in growth kinetics of viruses within the same subgroup. These studies demonstrate that subtle alterations in the FeLV SU, particularly in the N-terminal region, impart multiple significant functional differences which distinguish virus variants.

Exogenous feline leukemia viruses (FeLVs) have been classified into three subgroups (A, B, and C) by superinfection interference (52, 53). FeLVs induce a diverse spectrum of lymphoreticular and hemopoietic diseases in cats, and there is evidence that disease type may correlate with the FeLV subgroup(s) present in the infected animal (17, 30). Interference subgroup (53) and in vitro host range (23) are considered to be determined by the portion of the envelope gene that encodes the surface glycoprotein (SU). Subgroup A FeLVs (FeLV-A) are ecotropic, horizontally transmitted, present in all natural infections (22, 24), and can induce long-latency lymphoma. The FeLV-Glasgow-1-A isolate (FGA) (59) is the only molecularly cloned FeLV shown by the criterion of interference to belong to the A subgroup. Subgroup B FeLVs (FeLV-B) exhibit an extended host range in vitro (51, 54) and always occur in association with FeLV-A. Subgroup B viruses are formed by recombination between the FeLV-A *env* gene and endogenous provirus-like elements, giving rise to the FeLV-B *env* gene (37, 38, 59). FeLV-Gardner-Arnstein-B (FGB) and FeLV-Rickard-B (FRB) are the only molecularly cloned FeLV-B isolates classified by interference (12, 28). Subgroup C FeLVs (FeLV-C) (22) are also only found in association with

FeLV-A and are associated with fatal erythrocyte aplasia (19, 21, 33, 46). As with FeLV-B, FeLV-C isolates exhibit broadened host range and are unique in their ability to infect guinea pig fibroblasts (51, 54). FeLV-Sarma-C (FSC) is the molecularly cloned prototype (46).

An immunodeficiency disease-inducing FeLV isolate (FeLV-FAIDS), originally classified as a mixture of FeLV-A and -B (18, 29), was found to consist of subgroup A-like "common form" viruses (clone 61E is the prototype) and a family of immunodeficiency disease-associated variants (clone 61C is the prototype). Molecular clone 61C, although replication defective, could propagate in the presence of 61E as helper virus, and with 61E was used to construct a replication-competent chimera, designated EECC. 61E/61C mixtures and the chimera EECC cause T-cell killing in vitro (6) and induce immunodeficiency disease in vivo (6, 35, 41). T-cell killing by 61E/61C virus mixtures or chimeras is associated with massive superinfection and is preventable postinfection by adding virus neutralizing antiserum to block superinfection (6).

Although FeLV-A isolates have generally been found to be slowly pathogenic, their presence in all natural infections suggests that FeLV pathogenesis may involve interactions between multiple viruses, a concept first suggested by the work of Jarrett et al. (21). Although interference among oncoviruses has long been established, the possible interplay between coinfecting virus variants in the development of disease remains to be elucidated. For example, the manner in which Moloney murine leukemia virus and a mink cell

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FIG. 1. Virus chimeras, growth properties, and effects on cell killing by FeLV-EECC. Chimera designations (left) refer to the origin of sequences between the *XhoI* and *SacII* restriction sites, roughly corresponding to the SU-coding sequence. Letters refer to the virus of origin (E for 61E, S for FSC, and G for FGA; see Table 1); numbers at the bottom refer to the seven regions that compose this portion of the viral genome, delineated by restriction endonuclease sites or PCR primer sites (P1 and P2). Each amino acid that differs from that found in 61E is shown with a vertical line; the shaded boxes correspond to V1, V4, and V5 and indicate clusters of multiple changes (see Fig. 2 for details). Virus chimera growth kinetics are characterized as fast, which corresponds to an average of 2 to 3 weeks after infection for a culture to be scored as ELISA positive, or slow, corresponding to an average of 5 to 6 weeks for a parallel culture to be scored as ELISA positive. The effect of prior infection with the virus chimera shown on cell killing caused by subsequent infection with the cytopathic chimera EECC is shown in the far right column. The sequence of the first 163 bp of region 1 from FGA, corresponding to the *pol* gene segment, had not been reported previously but was sequenced in this study, and no nucleotide or amino acid changes relative to the 61E sequence were found (data not shown).

focus-forming virus interact to induce cytostatic effects on murine bone marrow cells and fibroblasts (27) is not well understood.

We have therefore sought to reexamine the process of superinfection and to examine the biochemical and genetic determinants within FeLV SU that mediate infection and interference.

MATERIALS AND METHODS

Viruses. The FeLV strains used in this study, all derived from molecularly cloned proviruses, were as follows: 61E (5, 35), FGA (59), FGB (28), FRB (12), FSC (46), 61E/61C chimeras, and the 61E/FSC (6, 34) chimeras EE[ES]E (45) and EE[ESE]E (2). Vesicular stomatitis virus (VSV)-FeLV pseudotypes [VSV(FeLV)] were generated by using VSV ts045, an Indiana strain harboring a mutant exterior envelope glycoprotein that is thermolabile and temperature sensitive for synthesis (13).

Generation of FeLV recombinant viruses. The nomenclature system used to describe FeLV virus chimeras is illus-

trated in Fig. 1 and Table 1. In all cases, chimeric envelope genes were cloned into a 61E provirus background of *gag*, *pol*, and transmembrane protein (TM)-long terminal repeat (LTR) sequences. Each chimeric virus will be referred to as chimera [1234567], where the bracketed region represents the seven regions encompassing the SU coding sequence that were interchanged; these include 163 bp at the 3' end of *pol* (region 1), the *env* leader sequence (region 2), and the SU (or gp70)-coding region of *env* (regions 3 to 7). A portion of the TM (or p15E)-coding region of *env* is found in region 7 but does not differ between the clones examined. Consistent with this nomenclature, EE[ES]E will be referred to as chimera [EEEESSS], EE[ESE]E will be referred to as chimera [ESSEEEE], and 61E will be referred to as chimera [EEEEEEEE].

Standard recombinant DNA techniques of restriction endonuclease fragmentation and ligation were used to construct most chimeric proviruses. However, because of a lack of the required restriction sites, chimeras [EEEESEEE], [EEEEEESE], and [EEEEEGGG] were generated by a polymerase chain reaction (PCR)-based cloning method similar

TABLE 1. Nomenclature used to define FeLV chimeras^a

Virus strain	Cloned virus	Subgroup	Env sector designation	Recombinant virus used ^b	Shortened virus designation
FeLV-FAIDS	61E	A ^c	E	EE[EEEEEEE]E	[EEEEEEE]
Glasgow-1-A	FGA	A	G	EE[GGGGGGG]E	[GGGGGGG]
FeLV-FAIDS	61C/EECC	A-like ^c	C	EE[CCCCCCC]E	[CCCCCCC]
Rickard-B	FRB	B			
Gardner-Arnstein-B	FGB	B			
Sarma-C	FSC	C	S	EE[SSSSSSS]E	[SSSSSSS]

^a The biological strain of origin and each molecularly cloned feline leukemia virus used in this study is listed by its three-letter designation as follows: 61E (5, 35) and 61C (and its replication-competent derivative, EECC) (35), both derived from the FeLV-FAIDS isolate (18); the subgroup A FGA derived from the Glasgow-1 isolate (59); the subgroup C FSC (46) derived from the Sarma isolate (53); and the subgroup B isolates FGB (28) and FRB (12), derived from the Gardner-Arnstein (14) and Rickard (44) strains, respectively.

^b The three letters outside the bracketed region refers to the 61E origin of *gag*, *pol*, and TM-LTR regions, respectively.

^c Subgroup determination made in this study.

to the overlap extension method (20). A series of six primers was generated; primers A (5'-CTTCAAACAGGAGACTC CGTCTG; the 3' end is base 5789 in the 61E genome [5]) and B (5'-GGCACTAATTGCTCTTCTA; the 3' end is complementary to base 7464) flanked the SU-coding sequence, whereas primers C and D and primers E and F were complementary pairs which annealed to conserved sequences between the regions of interest (C = 5'-ACATACC TAGCCTTAAATGCCA [base 6890], D = 5'-TGGCCATT TAAGGCTAGGTATGT, E = 5'-TGTCTTAATCGAAT TATGG [base 7246], and F = 5'-CCATAATTCGATTAA GACACAA). The appropriate primer pairs were annealed with DNA from chimeras and subjected to 11 cycles of PCR (94°C for 1 min, 56°C for 1 min, and 72°C for 1 min) in a 0.1-ml reaction mixture containing 100 pM each primer, 1 µg of target plasmid DNA, 2.5 U of *Taq* polymerase, and buffer specified by the enzyme manufacturer (Perkin Elmer-Cetus).

Fragments were separated by electrophoresis in 1% agarose; bands of interest were excised and centrifuged through Costar Spinex tubes for 15 min in a microcentrifuge. One microliter of eluate from each appropriate overlapping fragment (see below) was mixed and placed into a PCR reaction without primers and subjected to five cycles of PCR (94°C for 1 min, 54°C for 1 min, and 72°C for 1.5 min). Outside primers (A and B) were then added, and 35 more cycles of PCR were completed (15 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1.5 min followed by 20 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 2 min). Chimera [EEEEEGG] was generated by using the [EEEEEEE] A/D and [GGGGGGG] B/C amplification products, and [EE EESEE] was generated by using the [EEEESSS] A/D and [EEEEEEE] B/C products. Chimera [EEEEESE] was generated from an intermediate ([EEEEESSE]) made by using the [EEEESSS] A/F and [EEEEEEE] E/B products and finally by using the [EEEESE] B/C and [EEEEEEE] A/D products.

Following PCR, the samples were purified by ammonium acetate-room temperature ethanol precipitation (49), washed with cold 70% ethanol, dried, and subsequently cleaved with *Xho*I and *Sac*II. Fragments were separated by electrophoresis in low-melting-point agarose, and the fragments were excised and melted in a 65°C water bath; 10 µl of the resulting solution was used directly in a 20-µl ligation reaction (49) with a 3' 61E provirus subclone cleaved with *Xho*I and *Sac*II. Each fragment generated by PCR was sequenced (50) to confirm that no point mutations were introduced in the cloning procedure. The structure of each subsequent complete provirus clone was confirmed by restriction enzyme digestion and agarose gel electrophoresis

prior to transfection into mammalian cells. Furthermore, Southern transfer and hybridization with the probe exU3 (31) or PCR amplification of the envelope gene followed by restriction fragment length polymorphism analysis was used to confirm that cells were infected with the expected virus chimeras.

Cell lines. D17 is a canine osteosarcoma cell line (clone c1; ATCC CCL183), FeF is a feline embryo fibroblast cell line (AH927; originated by W. Nelson Rees and provided by S. Rasheed), and GP104 is a chemically transformed embryonic guinea pig fibroblast cell line (ATCC CRL1405, 104C1). Each cell line was maintained in Eagle's minimal essential medium or Dulbecco's modified Eagle's medium supplemented with penicillin, streptomycin, and glutamine (all from GIBCO) and 10% fetal bovine serum (FBS; HyClone). MLF, a mink lung fibroblast cell line (ATCC CCL64), was maintained in the media described above with 5% FBS. 3201B, a feline CD4⁺ CD8⁺ T-lymphoma cell line originated and provided by W. D. Hardy, Jr. (47, 57), and hereafter referred to as FeT cells for descriptive purposes, was maintained in 50% L15 (GIBCO)-50% RPMI 1640 (Hazelton or Sigma) supplemented with penicillin, streptomycin, glutamine, and 20% FBS.

Antibodies and Antisera. C11D8 is a murine monoclonal antibody shown to have neutralizing activity directed against all of the strains of FeLV used in this study (16) (see Results). The polyclonal sheep anti-VSV serum used in this study was kindly provided by Alice Huang. A 1/10 dilution was determined to neutralize 3.1 log units of a VSV stock possessing a titer of 1.2×10^8 in 40 min at 4°C.

Transfection. DEAE-dextran-mediated transfection of FeT and MLF cells was performed as described previously (8). Electroporation of FeT (10^7 early-log-phase cells in 0.4 ml of RPMI containing 0.1 mM dithiothreitol) and MLF (3×10^6 cells, 0.3 ml of Eagle's minimal essential medium) was conducted in serum- and antibiotic-free medium plus 20 to 30 µg of viral DNA at 250 V and 960 µF.

Flow cytometry and cell sorting. Flow cytometry and fluorescence-activated cell sorting (FACS) were done on an Ortho Diagnostics Cytofluorograf system 2150 equipped with a Data General 2151 computer and a 488-nm argon laser (Lexel). FeLV-infected FeT cells were labeled with C11D8 and fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Amersham) and sorted for maximum viral surface antigen expression. Cells with the 5% highest degree of fluorescence were collected, expanded, and used as the source of virus in the growth kinetics experiments described below.

Virus titration and analysis of FeLV growth kinetics. FeLV

stocks were titered in quadruplicate by limiting threefold serial dilution on either FeF or FeT cells. FeT cell cultures were split 1:15 once per week and were scored for virus production, assessed by detection of FeLV Gag p27 by enzyme-linked immunosorbent assay (ELISA) (Synbiotics) after 4 weeks for FeLV-C and 61C-LTR-containing viruses and after 6 weeks for all other viruses. FeF cell cultures were split 1:15 once every 5 days and were scored after 2 to 3 weeks. Titers are expressed as 50% tissue culture infectious dose and were calculated according to the method of Reed and Muench (42).

Virus growth kinetics were determined by measuring the time required for a low-multiplicity infection to give a positive ELISA signal. Threefold serially diluted virus stocks were plated in quadruplicate FeT cultures containing 10^5 cells in 1 ml. Cells were split 1:15 once per week after each ELISA test and were maintained for 7 weeks. By definition, the last sample to become ELISA positive represents a multiplicity of infection (MOI) of 10^{-5} .

FeLV-A-infected FeT cells were used as targets for EECC virus superinfection experiments. These cells were generated by expansion of the top 5% of virus envelope-expressing cells selected by FACS. The EECC virus stocks used reproducibly induced growth retardation (assessed by viable cell counts) and cell killing (assessed by trypan blue staining) in FeT cells approximately 8 days postinfection. EECC was inoculated onto log-phase FeT cell cultures containing 3×10^5 cells per ml; viable cells and trypan blue-staining cells were scored at day 4 and at roughly 2-day intervals thereafter until days 11 to 13, at which time most cultures had undergone cytopathic depletion.

For analysis of host range of EECC and derivative chimeras, 3×10^6 exponentially growing FeF cells were electroporated (960 μ F, 200 V) in 400 μ l of serum-free Dulbecco's modified Eagle's medium with 7 to 10 μ g of plasmid DNA containing an FeLV provirus. Culture supernatants (approximately 100 μ l) were analyzed for FeLV Gag p27 by antigen capture ELISA every 4 days for 28 days.

Preparation of VSV(FeLV) pseudotypes. D17 and MLF cells were initially infected with FeLV-containing transfected cell supernatants in the presence of DEAE-dextran (48) and maintained until they became strongly ELISA positive. To generate pseudotypes, a late-log-phase culture of these cells was superinfected with VSV at an MOI of approximately 30. Infection was allowed to proceed for 30 min at 22 to 25°C followed by 30 min at 37°C. Unattached virus was removed by aspiration and three washes with phosphate-buffered saline (PBS; GIBCO) before the addition of 3 ml of medium. Cells were then incubated for 6 to 12 h at 38.4°C (MLF cells) or 39°C (D17 cells). The supernatant was then removed and centrifuged for 20 min at 1,500 rpm in a Beckman TJ-6 centrifuge to remove cell debris. For assay of VSV or VSV pseudotype production, 300 μ l of supernatant was incubated for 1 h at room temperature with either 300 μ l of PBS or 300 μ l of a 1/15 dilution of anti-VSV antiserum, respectively. Tenfold serial dilutions of these samples were used to infect target cells in either the direct or indirect plaque assay.

Direct plaque assay. FeF and GP104 target cells were plated 24 h prior to assay at 450,000 cells per well in six-well (35-mm-diameter) plates. D17 cells were plated at 950,000 cells per well. Initial cell number was tripled when the assay was performed in 60-mm-diameter plates. Medium was aspirated from target monolayers and replaced with 200 to 300 μ l of the diluted pseudotype stock. Plates were then incubated for 45 to 60 min at 37°C before virus was removed

and the monolayers were overlaid with 3 ml of agar mix (0.9% agar, $1 \times$ Eagle's minimal essential medium, 4% FBS, 4% iron-saturated calf serum [HyClone], penicillin, streptomycin, gentamicin [GIBCO]). The agar overlay was allowed to set at room temperature, and the cultures were then incubated at 31 to 34°C for 40 to 48 h for FeF and D17 cells or 72 h for GP104 cells. Monolayers were fixed for 20 min in 3 ml of 37% formaldehyde, agar overlays were removed, and monolayers were stained with 1% methylene blue for 3 min. Plates were rinsed with water, dried, and scored for plaques. Pseudotype plating efficiency on the 10^{-1} dilution plate was slightly inhibited relative to the 10^{-2} dilution (~ 2 - to 5-fold lower than expected by extrapolation); therefore, whenever possible, the higher dilution was scored.

Indirect plaque assay. To examine infection of VSV (FeLV) pseudotypes in the nonadherent lymphoid FeT cell line, we developed an indirect plaque assay. In this assay, 2×10^6 FeT cells were pelleted and then resuspended in 100 to 200 μ l of undiluted pseudotyped virus stock. This mixture was incubated for 30 min at room temperature followed by 30 min at 37°C. The cells were then washed three times with 15 ml of PBS (GIBCO), resuspended in 1 ml of PBS, serially diluted in 10-fold increments into PBS, and plated onto D17 or FeF monolayers. Cells were allowed to settle onto the monolayer for 20 min at room temperature before the agar overlay was carefully added. Plates were then incubated and scored as in the direct plaque assay.

RESULTS

Development of a VSV(FeLV) pseudotype infection assay. FeLV subgroups have previously been defined by the focus interference assay (52, 53). We used an alternative technique developed by Zavada (62, 63), the pseudotype interference assay, in which VSV genomes are packaged within retrovirus envelopes and used to infect retrovirus-infected cells. Sommerfelt and Weiss (58) initially reported production of relatively low levels of VSV(FeLV) pseudotypes. We found that higher levels of VSV(FeLV) pseudotypes were produced by VSV superinfection of FeLV-infected D17 or MLF cells but were not detectable following VSV superinfection of FeLV-infected FeF or FeT cells (data not shown). We also found that the titers of VSV(FeLV), unlike most VSV (retrovirus) pseudotypes (58), were reduced to below detection levels by freezing (data not shown). We therefore expressed infectivity as the ratio of titer on a test cell line relative to titer on a standard, uninfected cell line examined in each experiment.

D17-derived VSV(FeLV-B) and VSV(FeLV-C) pseudotype stocks had average titers of 2.1×10^4 and 1.5×10^4 , respectively; however, D17-derived VSV(FeLV-A) pseudotype titers were over 10-fold lower. In contrast, VSV(FeLV-A, -B, and -C) titers of approximately 2×10^4 could be generated in MLF cells; hence, these cells were used for all experiments involving FeLV-A clones and their derivatives. The use of MLF cells resulted in an occasional low background level (usually one to three at the lowest dilution) of nonneutralizable plaques on FeF cells. These plaques may have been derived from pseudotypes that included envelope proteins from endogenous retroviruses present in MLF cells (1a, 25, 56) but were scored as VSV(FeLV) pseudotypes. Presence of these additional pseudotypes slightly reduced the magnitude of the receptor blockade scored (see below).

As a control to establish FeLV receptor-mediated infection blockade, we treated VSV(FeLV) pseudotypes with C11D8, a monoclonal antibody that binds the FeLV enve-

lope glycoprotein and has postbinding neutralizing activity against representatives of all three FeLV subgroups (15, 16). The C11D8 stock used neutralized >80% of the VSV(FeLV) pseudotypes derived from each of these three subgroups (Table 2). That EECC envelope glycoprotein binds to C11D8 (39, 43) and blocks the superinfection-associated cytopathic consequences of infection (6) strongly suggests that C11D8 neutralizes initial infection by EECC as well. Thus, nearly all of the pseudotypes gain entry to cells through utilization of an FeLV glycoprotein receptor.

Subgroup classification of molecularly cloned FeLV isolates. Interference was examined by plating VSV(FeLV) pseudotypes on FeF cells previously infected with viruses from each of the three FeLV subgroups. Plating efficiencies were either not reduced or reduced at least 1 and usually 2 to 3 orders of magnitude. The data shown in Table 2 are consistent with, and thus we adopted, the subgrouping convention established by Sarma and Log (52, 53) using a focus induction interference assay. Accordingly, FGA, FGB (and FRB), and FSC were considered prototype FeLV-A, -B, and -C isolates, respectively (2, 28, 46, 59). As seen in Table 2, both 61E (equivalent to chimera [EEEEEEE]) and the 61E/FSC chimera [EEEESSS] displayed mutual interference with the chimera [GGGGGGG], the latter of which derives its entire envelope gene from FGA. Thus, 61E and chimera [EEE ESSS] were classified as FeLV-A. FRB and FGB displayed mutual interference, as did FSC and chimera [ESSEEEE], confirming the latter as an FeLV-C isolate. VSV([EEEESSS]), VSV(FGB), and VSV(FSC) were used as control viruses to demonstrate that a series of chimeras, mostly derived from the subgroup A parental viruses FGA and 61E ([GGGGEEE], [EEEEGGG], [GGGEEEE], [EEEGEEE], [EEEEEGG], and [EEEESEE]), all behaved as subgroup A viruses (data not shown).

An infectious center indirect plaque assay was used to evaluate interference established in the nonadherent, feline T-cell line FeT (Table 3). The indirect plaque assay involves VSV(FeLV) pseudotype infection of FeT cells followed by exposure of these cells to an FeF cell monolayer for plaque induction. Receptor blockade in these cells was consistent with the direct plaque assay but was of a lesser magnitude, most likely due either to background mink cell-derived retrovirus pseudotypes or possibly to less efficient establishment of blockade in these cells. For example, FeLV-A-infected FeT cells were resistant to challenge by VSV (FeLV-A) pseudotypes within the limits of detection (40-fold loss in titer relative to uninfected FeT cells) but were normally infectable with subgroup C pseudotypes. Reciprocal results were obtained with VSV(FeLV-C) pseudotypes: superinfection was inhibited by prior infection with FeLV-C but not FeLV-A.

When VSV(EECC) pseudotypes were superinfected onto FeF cells preinfected with FeLV-B or FeLV-C isolates, no loss of titer was observed (relative to uninfected FeF cells). When these target cells were preinfected with 61E- or FGA-based FeLV-A strains, however, pseudotype infection was inhibited by 81 to >98% (Table 2). Because EECC does not infect FeF cells (see below), the reciprocal experiment could not be performed. However, when FeT cells that had recovered from an acutely cytopathic EECC infection were used as targets, VSV(FeLV-A) infectivity was reduced by about 60% or more (Table 3). These results reveal a partial interference between viruses bearing subgroup A- and 61C-derived SU proteins ([EECC] and 61E have identical transmembrane envelope proteins [35]).

Mechanisms of host cell restriction. FeLV interference

groupings have been shown to correlate with in vitro host range; FeLV-A isolates are usually ecotropic, that is, restricted to growth on feline cells, whereas FeLV-B isolates infect FeF and heterologous canine D17 osteosarcoma and human RD4 cells but usually fail to grow on FeT cells. FeLV-C isolates have the broadest host range, including FeF and FeT cells as well as human, dog, and guinea pig fibroblasts. To identify the levels at which host range restriction occurs, i.e., virus entry (attachment, penetration, and uncoating) versus postentry events, we conducted direct infection and VSV(FeLV) pseudotype infection experiments using uninfected FeF, FeT, D17, and GP104 cells as targets.

In direct infection experiments, titers of subgroup B viruses FGB and FRB were 10^4 - to 10^5 -fold lower on FeT cells than on FeF cells (Table 4, experiment A). The ratio of VSV(FeLV-B)-derived plaques produced in FeF versus FeT cells (the latter assayed indirectly as described above) was also greater than that produced by other VSV(FeLV) pseudotypes (Table 4, experiment B). Thus, a significant block to subgroup B replication in FeT cells occurred at the level of virus entry.

Consistent with direct infection studies (45), FeLV-B and FeLV-C pseudotypes both infected D17 cells (Table 5). VSV(FeLV-B) pseudotypes produced roughly equivalent numbers of plaques on FeF and D17 cells. VSV(FeLV-C) pseudotypes, by contrast, yielded ~10-fold more plaques on FeF cells. Similarly consistent with previous direct infection studies (45, 54), VSV(FeLV-C) pseudotypes infected GP104 cells, whereas VSV(FeLV-A) and VSV(FeLV-B) pseudotypes did not (Table 5). Thus, host range restriction of FeLV-A and -B on GP104 cells occurs at the level of virus entry.

Subgroup A VSV([EEEESSS]) pseudotypes yielded 20-fold or more plaques on FeF cells than on D17 cells (Table 5). However, these particles had greater infectivity for D17 cells than did VSV([GGGGGGG]) and VSV([EEEEEEE]) pseudotypes. This result is consistent with that of Riedel et al. (45), who showed that [EEEESSS] ([ES] in their nomenclature) grew in D17 cells, whereas [EEEEEEE] did not. Thus, there are at least two SU regions which determine host range, the N-terminal region as well as or in combination with C-terminal sequences.

VSV(EECC) pseudotypes readily infected and induced plaques on FeF cells (Table 2). However, EECC and other FeLVs bearing an SU principally derived from 61C failed to infect FeF cells as assessed by evidence of progeny virus production (Table 6). Using a series of 61C/61E chimeras (6), we found that sequences in the N-terminal regions of SU were principally responsible for this postentry level of restriction (Table 6), although combinations of changes downstream involving principally what corresponds to the sixth region (Fig. 2) resulted in restriction in some experiments. Furthermore, 3 weeks after infection of FeF cells with EECC at an MOI of 0.001, no virus was detectable by either p27 ELISA or Southern blot (Table 6), whereas cell supernatants could then be removed and passaged successfully onto uninfected FeT cells (data not shown). In contrast, a similar MOI of EECC infected onto FeT cells resulted in readily detectable virus by either assay within 1 week postinfection. These findings indicate that the 61C SU protein can mediate virus entry into FeF cells but that the quantitative impairment of virus spread in FeF cells is due to an envelope-associated, postentry level of restriction, principally as a result of changes near the N terminus.

Effect of gp70 variation on FeLV-A growth kinetics. To examine the role of the envelope protein in virus growth

TABLE 2. FeLV subgroups determined in a VSV(FeLV) pseudotype assay

VSV(FeLV) pseudotype	Titer (PFU/ml) ^a on FeF cells infected with:									Sub- group
	No virus	No virus + C11D8	61E	[EEEESSS]	[GGGGGGG]	FRB	FGB	FSC	[ESSEEEE]	
61E [EEEEEEEE]	+++	-	-	-	-	-	+++	+++	-	A
	1	0.04 (1)	<0.003-0.029 (4)	<0.1 (1 ^b)	<0.1 (1 ^b)	ND	1.1-2.0 (2)	0.81-0.94 (2)	ND	
[GGGGGGGG]	+++	-	-	-	-	ND	+++	+++	ND	A ^c
	1	<0.2 (1 ^c)	0.003-0.006 (3)	<0.006-0.06 (2)	<0.006-0.06 (2)	ND	1.4-1.7 (2)	1.1 (2 ^d)	ND	
[EEEESSSS]	+++	-	-	-	-	ND	+++	+++	+++	A
	1	<0.03-0.04 (2)	0.002-<0.025 (4)	<0.019-0.025 (3)	<0.02-0.05 (2)	ND	1.4-3.4 (2)	0.74-0.8 (2)	0.77-3.4 (2)	
EECC	+++	ND	-	+	-	ND	+++	+++	ND	A ^c
	1	ND	0.03-0.19 (2 ^e)	0.07-0.10 (2 ^e)	<0.02-0.20 (2 ^b)	ND	1.4-1.5 (2)	1.0-1.1 (2)	ND	
FRB	+++	-	+++	+++	-	<0.016-0.02 (2)	-	ND	+++	B ^c
	1	0.02 (1)	0.96-0.99 (2)	0.99 (1)	ND	<0.016-0.02 (2)	<0.016-<0.03 (2)	ND	0.66-0.82 (2)	
FGB	+++	-	+++	+++	ND	-	-	ND	+++	B ^c
	1	<0.04 (1)	0.75-1.36 (2)	1.0 (2) ^f	ND	<0.002-<0.011 (2)	<0.002-<0.011 (3)	ND	0.95-1.0 (2)	
FSC	+++	ND	+++	+++	ND	+++	+++	-	-	C ^c
	1	ND	1.1-1.67 (2)	1.1-2.83 (2)	ND	1.66-2.17 (2)	1.13-1.67 (2)	<0.003-<0.017 (2)	<0.003-<0.017 (2)	
[ESSEEEEE]	+++	-	+++	+++	ND	+++	+++	-	-	C
	1	0.07 (1)	0.89-1.12 (2)	1.31-1.41 (2)	ND	0.88-1.05 (2)	0.60-2.05 (2)	≤0.004 (2)	<0.003-<0.005 (3)	

^a Ratio of titer on a given target cell versus titer on uninfected FeF cells. The range of values obtained is shown, and the number of experiments on which the range is based is given in parentheses. Actual numbers of plaques counted are provided for the values with footnotes. FRB, FGB, and FSC pseudotypes were generated in D17 cells; all other pseudotypes were generated in MLF cells. Plus and minus criteria for growth, assigned as specified below, are based on the greatest inhibition (low end of range) observed. This serves to minimize the effect of plaques arising from nonneutralized VSV- or endogenous MLF-derived viral pseudotypes (see text for discussion). Prototype FeLV subgroups were assigned on the basis of the results of focus induction interference assays as described in the introduction. Symbols: +++, >0.32; ++, 0.11 to 0.31; +, 0.035 to 0.1; - <0.035. ND, not done.

^b Ten plaques on uninfected cells versus no plaques on previously infected cells.

^c No plaques in the presence and five plaques in the absence of C11D8 antibody.

^d Totals of 20 and 19 plaques on FSC-infected cells versus 18 and 17 plaques on uninfected cells in the experimental replicates.

^e The parental viruses that contributed the gp70-coding sequence of these chimeras were previously shown by focus interference to belong to the subgroups indicated (see text).

^f Totals of 6 and 17 plaques on 61E-infected cells versus 20 and 96 plaques on uninfected cells in experimental replicates.

^g Totals of 3 and 9 plaques on [EEEESSS]-infected cells versus 41 and 88 plaques on uninfected cells in the experimental replicates.

^h Totals of 0 and 18 plaques on [GGGGGGG]-infected cells versus 41 and 88 plaques on uninfected cells in the experimental replicates.

ⁱ Shares some but not all features with the other two subgroup A viruses (see text for discussion).

^j Totals of 43, 30, and 30 plaques on FRB-infected cells versus 43, 30, and 34 plaques on uninfected cells in the experimental replicates.

TABLE 3. Indirect plaque assay of VSV(FeLV) pseudotype infection of FeT cells^a

VSV(FeLV) pseudotype (host cell)	Titer (PFU/ml) on FeT cells infected with:					
	No virus	61E	[EEEESSS]	[GGGGGGG]	EECC	[ESSEEEE]
[EEEEEEE] (MLF)	+++ 1	— 0.03 (1)	+	—	++ 0.16–0.21 (2)	ND
[GGGGGGG] (MLF)	+++ 1	— 0.03–0.05 (2)	—	—	++ 0.16–0.33 (2)	ND
[EEEESSS] (MLF)	+++ 1	— <0.03–0.12 (2)	— <0.03 (2)	— <0.03 (2)	++ 0.21–0.41 (2)	+++ 0.9–1.08 (2)
FSC (D17)	+++ 1	+++ 1.19 (1)	+++ 1.13 (1)	+++ 1.08 (1)	+++ 0.7–0.95 (3)	— <0.02–0.18 (3)

^a For details, see Table 2, footnote a. FSC pseudotypes were generated in D17 cells; all other pseudotypes were generated in MLF cells.

kinetics, we measured the time required for an FeLV-A infection at an MOI of 10^{-5} to generate p27^{gag} detectable by ELISA in FeT cell cultures. Results from a representative experiment are summarized in Fig. 1. Whereas [EEEEEEE] required 6 weeks to generate detectable p27 (slow growth kinetics), replication of the *env* chimeras [GGGGGGG] and [EEEESSS] was evident by 3 weeks (fast growth kinetics). Analysis of a panel of chimeras in this way permitted localization of the amino acid changes that accelerated viral growth to three regions. Four amino acid changes near the N terminus of 61E (chimera [GGGEEEE]) were sufficient to enhance growth; one or two amino acid changes in the C-terminal half of the 61E protein (compare chimeras [EE EGGG] and [EEEEEGG]) were also sufficient, as was substitution of the fourth variable (V4) region of the envelope protein from FSC (chimera [EEEESEE]). Interestingly, the single amino acid change (Val to Met) clearly implicated as an FGA C-terminal determinant was also found in the FSC V4 region (Figs. 1 and 2). Thus, SU-mediated determinants for the rapid growth phenotype have been identified in both the N-terminal and central regions of the protein.

EECC superinfection and killing of FeLV-A-infected FeT cells. As cloned directly from tissues, highly pathogenic FeLV-FAIDS viral genomes (61C is the prototype) have been replication defective and thus have required helper viruses (61E, cloned from the same cat tissue, is the prototype) for transmission (35, 36). We have therefore explored the interactions that occur as a result of dual virus infection

in cells by studying the effect of prior infection with the noncytopathic subgroup A virus chimeras [EEEEEEE], [GGGGGGG], and [EEEESSS] on the growth and cytopathicity of 61C, represented by the chimera EECC.

Prior infection of FeT cells with virus chimera [EEEE EEE], [EEEESSS], or [GGGGGGG] either had no effect on or increased viable cell number in comparison with uninfected controls (data not shown). In contrast, EECC infection of naive FeT cells resulted in cell clumping (a hallmark of EECC infection) (data not shown) and the onset of cell killing within 4 to 6 days (Fig. 3A and C). Cell clumping in these cultures was unaffected by prior infection with FeLV-A viruses, suggesting that the kinetics of EECC virus infection was unaffected by the presence of FeLV-A. However, prior infection with FeLV-A chimera [GGGGGGG] did have an effect on cell growth retardation and killing caused by EECC, the determinants of which were mapped to multiple changes principally found in the N-terminal portion of the SU protein (Fig. 3B and D). Chimera [GGGEEEE] had nearly the same effect as [GGGGGGG], whereas chimeras [GGGEEEE] and [EEEGEEE] produced intermediate effects (Fig. 3B and D; summarized in Fig. 1).

Cell killing and growth retardation observed in infected and uninfected target cells was quantitatively assessed by analysis of infections initiated with threefold serial dilutions of EECC (Table 7). When cell killing was assessed, prior infection with chimera [GGGGGGG] was found to be equivalent to initiating the infection with 40-fold less EECC,

TABLE 4. Infectious properties of FeLV-B isolates on FeF and FeT cells

Expt	Isolate	Subgroup	Titer on FeF Cells	Relative titer (PFU, direct/indirect) ^a	No. of expts
A ^b	FRB		9.0×10^4		1
	FGB		1.6×10^5		2
B ^c	[EEEEEEE]	A		11–71	3
	[EEEESSS]	A		23–188	3
	[GGGGGGG]	A		9–137	3
	EECC	A*		19–153	2
	FRB	B		428	1
	FGB	B		700–19,800	2
	FSC	C		15–45	4

^a Ratio of the number of plaques observed in the direct plaque assay for each one observed in the indirect assay on FeT cells. Direct plaque assays were conducted on FeF cells except for the FGB replicate, which yielded a titer of 700:1 in a direct plaque assay in D17 cells. As discussed in the text, VSV(FRB) pseudotypes had approximately equal titers on D17 and FeF cells.

^b Relative infectivity of FeLV-B isolates on FeF cells, reported per milliliter of cell supernatant and based on analyses of limiting dilutions of a given virus. No infectivity was found in FeT cells.

^c Relative titer of VSV(FeLV) pseudotypes in direct and indirect interference assays.

TABLE 5. Host range of VSV(FeLV) pseudotypes^a

VSV(FeLV) pseudotype (host cell)	Titer on indicated uninfected target cell		
	FeF	D17	GP104
[EEEEEEE] (MLF)	+++ 1	— <0.001–<0.02 (4)	— <0.005–<0.02 (2)
[GGGGGGG] (MLF)	+++ 1	— 0.002–<0.2 (2)	— <0.2 (1)
[EEEESSS] (MLF)	+++ 1	— 0.025–0.05 (3)	— <0.03–<0.05 (2)
FRB (D17)	+++ 1	+++ 0.5–4.18 (4)	— <0.04 (1)
FGB (D17)	+++ 1	+++ 0.94–6.67 (4)	— <0.002–<0.01 (2)
FSC (D17)	+++ 1	++ 0.15–0.23 (2)	ND
[ESSEEEE] (D17)	+++ 1	+ 0.06–0.13 (2)	++ 0.12–0.19 (2)

^a Titer is reported as the ratio of titer on a given target cell versus titer on uninfected FeF cells. The range of values obtained is shown. The number of experiments on which the range is based is given in parentheses. VSV(FeLV-A) pseudotypes were generated in MLF cells; VSV(FeLV-B) and VSV(FeLV-C) pseudotypes were generated in D17 cells. Symbols, abbreviation, and criteria are the same as for Table 1.

whereas prior infection with subgenetic chimeras [GGG GEEE] and [GGGEEEE] approximated 20- and 7-fold less input EECC virus, respectively. When cell growth was assessed, analysis of viable cell counts indicated that prior infection with chimera [GGGGGGG] was equivalent to initiating infection with ninefold less EECC, and threefold less as a result of prior infection with [GGGGEEE].

TABLE 6. FeLV 61E/61C chimera propagation in FeF cells^a

Plasmid	Propagation on FeF cells	FeT cell killing
EE[EEEE]C	+	—
EE[EEEC]C	+	—
EE[EECE]C	+	+
EE[ECEE]C	+	—
EE[ECEC]C	+	—
EE[ECCE]C	+	++
EE[EECC]C	+/-	++
EE[ECCC]C	+/-	++
EE[CEEE]C	—	Defective
EE[CCEE]C	—	Defective
EE[CCEC]C	—	++
EE[CEEC]C	—	++
EE[CECC]C	—	NT
EE[CECE]C	—	+++
EE[CCCE]E	—	+++
EE[CCCC]C	—	+++

^a Plasmids containing FeLV provirus were electroporated into FeF cells, and virus propagation was analyzed for FeLV Gag p27 ELISA. Results are represented as follows: +, ELISA positive beginning 4 to 8 days postelectroporation (PE); +/-, ELISA positive beginning not earlier than 16 days PE in three experiments and negative in most experiments; +/—, ELISA positive beginning day 20 PE in one experiment and negative in four experiments; —, no detectable ELISA signal by 28 days PE. Classifications of in vitro T-cell cytopathicity are taken from Donahue et al. (6). Electroporations were performed a minimum of two times with each plasmid. SU-coding sequences are divided into four sectors here rather than seven since these chimeras were generated in a previous study (6). See Fig. 2 for sector borders and descriptions. NT, not tested.

DISCUSSION

Previous studies demonstrated the importance of FeLV SU proteins in determining disease type specificity, the kinetics and efficiency of disease induction in vivo, and cell killing in vitro (6, 36, 41, 45). Our goal in this study was to begin dissection of the distinct virologic properties imparted by SU proteins from biologically distinct FeLVs.

FeLV-FAIDS clone 61E had been tentatively classified as FeLV-A because of the close homology of its envelope gene sequence to that of FGA and its generally ecotropic host range (5). We have now formally classified 61E as a subgroup A FeLV on the basis of pseudotype interference in FeF and FeT cells. Chimera [EEEESSS] retained the FeLV-A phenotype, as judged by its reciprocal interference with chimera [GGGGGGG], thus localizing the subgroup A-determining region sequences 5' to the *Bcl*I restriction site in the SU-coding domain of *env* (Fig. 2). The mutual interference of chimera [ESSEEEE] with FSC more finely localized a FeLV-C subgroup determinant to 5' of the *Dra*III site (Fig. 2). VSV pseudotypes bearing envelope genes from three additional FeLV-C isolates (FZ215, FZ246, and FA27 [33]) have also been used recently to demonstrate that the subgroup C determinants colocalize to region 3 of SU (2).

Dorner and Coffin (7) localized receptor-determining regions of the avian leukosis virus (ALV) SU protein to two regions in the middle third of gp85. The murine retrovirus 10A1, which is inferred to utilize two separate receptors, has been interpreted to have three receptor-determining sites, near positions 12, 30, and 63% along SU (34). Thus, whereas amino acid changes that specify FeLV-A/FeLV-C receptor choice are localized to a small region of SU, both the avian and murine virus studies demonstrate that combinations of sequences from more than one region can be involved in determining receptor specificity.

In contrast to FeLV-A and FeLV-C, neither of two FeLV-B clones nor VSV(FeLV-B) pseudotypes were found to infect FeT cells. FeT cells may lack some factor, for

FIG. 2. Amino acid alignments of FeLV SU proteins. Alignments are broken into regions corresponding to those shown in Fig. 1, with only regions 2 to 7 shown. Amino acids are replaced with dot when the same as those found in 61E. In a previous study (6), chimeras generated between 61E and 61C were divided into four sectors and used for the studies reported in Table 6. These sector borders and identities are shown below each aligned region. Potential N-linked glycosylation sites are underlined.

example, a cell surface receptor, that is necessary for FeLV-B penetration into these cells. Alternatively, the low levels of endogenous FeLV envelope mRNA produced in these cells (27a, 31a, 38) may encode an SU-like protein sufficiently abundant and available to block or downregulate its cognate receptor. Consistent with this hypothesis is the finding that some substrains of this cell line are capable of supporting FeLV-B infection (17a). Subgroup B viruses are likely to be most interfered with by such expression, since they derive their unique SU determinants through recombination with endogenous FeLV provirus-like sequences (37, 59). The monoclonal antibody C11D8 (11, 16) binds to and blocks infection by the two FeLV-B isolates examined here. Its epitope sequence is found within feline endogenous FeLV-related sequences (26, 27a), yet it fails to bind to the surface of uninfected FeT cells, suggesting that if the endogenous viral protein is produced and the C11D8 epitope is appropriately expressed (32), the endogenous FeLV protein may interfere by blocking the receptor from reaching the cell surface. This hypothesis would be consistent with the receptor downregulation observed through study of avian reticuloendotheliosis viruses (4).

We found some discordance between host range and interference properties within the subgroup A viruses. Although poorly infectious, VSV([EEEESSS]) pseudotypes were found to be approximately 15-fold more infectious for D17 cells than were VSV([EEEEEEE]) pseudotypes, consistent with the poor horizontal transmissibility of 61E on these cells (45). In agreement with the early observations of

Jarrett et al. (23) and Sarma et al. (51, 54), we conclude that growth or restriction of FeLV in D17 and GP104 cells correlates with the ability of the SU protein to affect virus entry. The differences between [EEEEEEE] and [EEEESSS] SU proteins may result in dualtropic utilization by the latter protein of a receptor on D17 cells that is distinct from the subgroup A receptor that it recognizes on feline cells. Alternatively, it may also result from an enhanced ability to bind a common receptor present on D17 cells.

In contrast, since VSV(EECC) pseudotypes can readily enter FeF cells, the restriction for EECC growth on these cells must occur as a result of postentry events. This restriction is likely to occur at some posttranslational stage of virus production and assembly, since a low level of infectious virus can be recovered from FeF cells. Indeed, the 61C envelope protein, relative to that of 61E, has been shown to be expressed at elevated steady-state levels, to be differentially glycosylated, to form an antigenically distinct SU-TM complex, and to be posttranslationally cleaved more slowly in these cells (39, 40). Related phenomena have been observed in other retrovirus systems. For example, baboon endogenous virus (BEV) and the endogenous xenotropic feline virus RD114 have identical host ranges and belong to the same interference group (55), yet the mechanisms of growth restriction of these viruses in mouse and rat cell lines differ. VSV(BEV) pseudotypes infected both cell types, implying that restriction of BEV growth was due to postentry events, whereas VSV(RD114) pseudotypes did not infect

FIG. 3. Cytopathic effects induced by EECC in uninfected and FeLV-A-infected target cells. (A and B) Total numbers of viable cells are represented by a plot of the logarithm of the cumulative viable cell number increase. (C and D) Percent trypan blue-positive (nonviable) cells are given at the indicated days postinfection. Both sets of curves are from representatives of three to four separate experiments with each virus chimera. Replicates are shown in the case of EECC infection of uninfected and [GGGGGGG]-infected FeT cells.

these cells, suggesting that RD114 is blocked at the level of virus entry (55).

FeLV-A virus chimeras were also found to differ in their growth kinetics. For example, chimera [EEEESEE] displayed fast growth kinetics, while [EEEEEEE] and [EEE EESE] displayed slow growth kinetics (Fig. 1). Thus, changes in the fifth region of gp70 can accelerate growth kinetics. Chimeras [GGGGEEE] and [EEEEGGG] both had fast growth kinetics; thus, chimera [GGGGGGG] appears to have changes in two independent regions within the envelope gene that enhance growth kinetics. The N-terminal determinant localizes to four amino acid changes 5' of the *Dra*III site. The C-terminal determinant localizes to one or two amino acid changes. The first of these, a valine-to-methionine change, is also present in the critical sector of the fast-growing chimeras [EEEESSS] and [EEEESEE], whereas the second change by itself was found to have no effect on growth kinetics.

In summary, our results show that subtle mutational changes in FeLV envelope proteins can be responsible for receptor specificity, host range, and, as separately deter-

TABLE 7. FeLV-A inhibition of EECC-induced cytopathicity^a

Preinfecting virus envelope	Inhibition of cell killing					Inhibition of growth retardation				
	Expt				Geometric mean	Expt				Geometric mean
	1	2	3	4		1	2	3	4	
[EEEEEEEE]	0	0	0	0	0	0	0	0	0	0
[EEEESSS]	0	3	0	3	0	0	0	0	0	0
[GGGGGGG]	10	30	30	200	40	10	3	20	10	9
[GGGGEEE]	30	3	100		20	3	3	10		3
[GGGEEEE]	3	3	30		7	0	3	3		0
[EEEEEEEE]	3	0	6		0	0	0	3		0
[EEEEGGG]	3	0	3		0	0	0	0		0

^a Results are expressed as the fold less EECC required to initiate an equivalent infection on uninfected FeT cells (rounded to one significant digit). Viable and trypan blue-staining cells were counted at the first time point at which approximately 50% of naive, EECC-infected FeT cells stained with trypan blue.

mined by changes within the enhancer region of the viral LTR (6), kinetics of virus spread. Identification and study of the FeLV-A receptor (15), the FeLV-C receptor, and their cognate SU interactions should facilitate elucidation of the molecular mechanisms that give rise to these differences.

A series of studies that we performed had bearing on the analysis of receptor utilization and interactions between FeLV-A and EECC. We found that chimeras [EEEEEEEE] and [GGGGGGG] potentially and reciprocally interfered with infection but less potently interfered with VSV(EECC) infection of FeT cells (Table 2). Furthermore, infection of FeT cells by EECC resulted in only weak interference of subsequent VSV([EEEEEEEE]) or VSV([GGGGGGG]) infection. FeLV-A strains also differed in the ability to interfere with cell killing and growth retardation caused by subsequent infection of FeT cells by EECC: [EEEEEEEE] failed to interfere, whereas chimera [GGGGGGG] partially interfered. Thus, although some entry-level interference with EECC occurred, postentry effects were likely to be more important for the observed inhibition of growth retardation and cell killing. Lastly, the determinants of cell killing interference by chimera [GGGGGGG] map to the same region of SU that determines subgroup A identity.

From the partial interference observed, it remains plausible that FeLV-A and EECC use the same single receptor. According to this hypothesis, the inefficient blockade of EECC infection and cytopathicity by FeLV-A could result from a lower affinity of FeLV-A than EECC for the receptor or from a reduction of the number of receptor molecules on infected cell membranes. Partial interference might then be sufficient to impair superinfection-mediated cytopathicity. However, it is also possible either that EECC employs a receptor in addition to the subgroup A receptor on FeT cells that provides a less efficient (as a result of low abundance or low affinity) portal of entry or that EECC employs both a common and a required secondary receptor, the latter of which would be distinct from that of the subgroup-specific secondary receptor for FeLV-A. We have shown elsewhere (43) that purified 61E and 61C proteins coprecipitate a 70-kDa cell surface protein, despite exhibiting distinct interference properties. Data suggesting that all FeLV subgroups employ a common initial receptor or docking protein but that subgroup specificity is provided by secondary, postbinding interactions at the cell membrane have recently been presented (1, 3). Lastly, if FeLV SU proteins, like those of other retroviruses, function within multimeric structures (10,

60), it is also conceivable that the heteromultimeric SU proteins that might form between EECC and FeLV-FGA SU proteins in coinfecting cells are more efficient at superinfection blockade than are SU homomultimers.

The observation that EECC can superinfect FeT cells infected with either homologous or subgroup A FeLV suggests that cell killing and disease induction by EECC might be related to the inability of some feline cells to prevent this superinfection. Indeed, 61C SU fails to establish superinfection interference to homologous virus challenge in the absence of virus replication (43). Analogy can be made to the study of ALV, for which a lack of or delay in the establishment of superinfection resistance leads to cell killing in cells infected with ALV-B and ALV-D (61). ALV-B and ALV-D display mutual interference, fail to establish complete receptor blockade, and induce cytopathicity correlated with an accumulation of unintegrated viral DNA (61). Use of the ALV-B receptor appears both necessary and sufficient to induce cytopathicity (7). ALV-B and ALV-D isolates also differ in host range; ALV-D grows in chick cells that lack the ALV-B receptor and are able to penetrate some mammalian cells (9). ALV-E isolates do not induce acute disease and do not prevent superinfection by ALV-B and ALV-D, even though superinfection by ALV-E is prevented by preinfection with ALV-B or ALV-D. Within the phenotypically heterogeneous A subgroup of FeLVs, then, are properties analogous to those that distinguish three ALV subgroups. Further dissection of FeLV-A isolates awaits isolation and analysis of their interactions with a cellular receptor(s).

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