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## Recombinant Feline Herpesviruses Expressing Feline Leukemia Virus Envelope and *gag* Proteins

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We constructed recombinant feline herpesviruses (FHV) expressing the envelope (*env*) and *gag* genes of feline leukemia virus (FeLV). Expression cassettes, utilizing the human cytomegalovirus immediate-early promoter, were inserted within the thymidine kinase gene of FHV. The FeLV *env* glycoprotein expressed by recombinant FHV was processed and transported to the cell surface much as in FeLV infection, with the exception that proteolytic processing to yield the mature gp70 and p15E proteins was less efficient in the context of herpesvirus infection. Glycosylation of the *env* protein was not affected; modification continued in the absence of efficient proteolytic processing to generate terminally glycosylated gp85 and gp70 proteins. A recombinant FHV containing the FeLV *gag* and protease genes expressed both *gag* and *gag*-protease precursor proteins. Functional protease was produced which mediated the proteolytic maturation of the FeLV *gag* proteins as in authentic FeLV infection. Use of these recombinant FHV as live-virus vaccines may provide insight as to the role of specific retroviral proteins in protective immunity. The current use of conventional attenuated FHV vaccines speaks to the wider potential of recombinant FHV for vaccination in cats.

Viruses of the family *Retroviridae* are known to cause a variety of infectious diseases of medical and veterinary importance. Despite an extensive literature in the area of retrovirus immunology, little is known about protective immunogens and protective immune responses in retroviral infection. Perhaps the best-studied retrovirus for which protective immunity has been sought is feline leukemia virus (FeLV). Infection in domestic cats by this type C oncornavirus is widespread, and the natural history of infection and disease has been the subject of numerous reviews (24, 58). Protection against FeLV disease is afforded by vaccination using whole inactivated virus (23, 27, 39, 53).

Efforts to define critical immunogens important for protective immunity against FeLV infection and disease have focused on the viral envelope (*env*) gene product. The FeLV *env* gene encodes a gp85 precursor protein which is proteolytically processed by cellular enzyme(s) to yield the major envelope glycoprotein gp70 and the associated transmembrane protein p15E. Virus-neutralizing antibody directed against gp70 may be important in protective immunity (7, 49). More recently, it has been appreciated that cell-mediated immune responses to internal viral proteins may also be important (1, 10, 26, 32, 37). The internal structural proteins of FeLV are encoded by the *gag* gene. Proteolytic processing of the Pr65<sup>gag</sup> precursor protein by the retroviral *pol*-encoded protease results in the formation of mature p15 matrix (MA), pp12, p27 capsid (CA), and p10 nucleocapsid (NC) proteins of the FeLV core. Expression of the *gag-pol* fusion protein (Pr180<sup>gag-pol</sup>) occurs by suppression of the *gag* termination codon by a cellular tRNA<sup>Glu</sup> (72). This precursor, upon autoprocessing by the *pol* protease, also encodes the viral reverse transcriptase and integrase.

We were interested to explore further the role of the retrovirus *env* and *gag* proteins in protective immunity. Because live-virus vaccines tend to elicit strong and durable

immunity involving both humoral and cellular arms of the immune system, we sought to develop a live-virus vector with which to present FeLV proteins to the immune systems of cats. We chose to investigate the use of the feline-specific herpesvirus, feline herpesvirus 1 (FHV), as a recombinant virus vector for vaccination in cats.

FHV is a member of the alphaherpesvirus subfamily and is the causative agent of feline viral rhinotracheitis (5, 25, 57). Feline viral rhinotracheitis vaccines comprising modified live or killed FHV are in widespread use and have been successful in reducing the incidence of disease (2, 55). The recent development of live recombinant herpesvirus vaccines, such as those to protect swine against pseudorabies disease (35, 45), suggested that recombinant FHV might also be useful in vaccination. These viruses might be engineered to express heterologous immunogens during vaccinal infection (40, 62, 68, 70). In this report, we describe the development of recombinant FHV expressing the *env* and *gag* proteins of FeLV.

### MATERIALS AND METHODS

**Cells and viruses.** The growth of FHV strain UT88 in feline kidney (CRFK) cells and the generation of recombinant thymidine kinase-negative (TK<sup>-</sup>) viruses has been described previously (50). FeLV subgroup A was derived from a molecularly cloned infectious provirus (pFGA-5) of FeLV-A/Glasgow (66). The provirus was introduced into CRFK cells, and a persistently infected cell line (FeLV-A/FGA) was obtained by passage. FeLV virions were prepared from clarified cell culture supernatants by centrifugation through 10% sucrose.

**Nucleic acid techniques.** The molecularly cloned infectious FeLV provirus pFGA-5 (66) was kindly provided by James Neil (Beatson Institute, Glasgow, Scotland). The promoter-insertion plasmid pON1 (64) was the kind gift of Ed Mocarski (Stanford University, Stanford, Calif.), and the molecularly cloned human cytomegalovirus immediate-early (CMV IE) gene promoter (3, 65) was provided by Walter Schaffner (University of Zurich, Zurich, Switzerland). Molecular cloning and other nucleic acid techniques were as described by

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Maniatis et al. (44). Nucleic acid enzymes were from New England BioLabs, Inc., Beverly, Mass., or Bethesda Research Laboratories, Inc., Gaithersburg, Md. Polymerase chain reactions (PCRs) using the thermostable DNA polymerase of *Thermus aquaticus* (Perkin-Elmer Cetus) were as described previously (59).

**Plasmid constructions.** The FeLV-A *env* gene from pFGA-5 (66) was subcloned as a *Pst*I fragment in pUC19 (pUC-FeLVenv). This FeLV fragment contains the entire *env* gene, including 61 nucleotides upstream of the *env* ATG codon and 90 nucleotides downstream of the *env* termination codon. No extraneous ATG codons precede the authentic *env* ATG codon on this fragment.

The CMV IE promoter was used as a *Pst*I to *Sac*II fragment which spans positions -1141 to +21 base pairs (3) and includes enhancer and regulatory regions. This fragment was inserted into the polylinker of the promoter-insertion vector pON1 (64), directly upstream of a modified *Escherichia coli*  $\beta$ -galactosidase gene. Simian virus 40 polyadenylation and enhancer regions are located downstream of the  $\beta$ -galactosidase gene. Insertion of the CMV IE promoter yielded the plasmid pON1-CMVIE. For expression of FeLV *env*, the  $\beta$ -galactosidase gene (*Xba*I to *Pvu*II) was replaced by the *Pst*I fragment containing FeLV *env*. The resulting plasmid was designated pON-CMVenv (pGC110).

The FHV TK deletion plasmid *ptk* $\Delta$ *EcoRV-Hind*III (pGC113) contains a 347-base-pair deletion between the *EcoRV* and *Hind*III sites in the FHV TK coding sequence (50). The *Hind*III site was regenerated by the synthetic oligonucleotide used to join the ends of the deletion. Another FHV TK deletion plasmid, *ptk* $\Delta$ *EcoRI-Hind*III (pGC111), bears a larger deletion in TK, from the *EcoRI* site in the putative 5' untranslated region of the TK gene through to the internal *Hind*III site described above. A synthetic, partially double-stranded oligonucleotide (5'AATTCGCGGCCGCA 3'/3'GCGCCGGCGTTCGA5') was used to join and regenerate these sites.

*EcoRI* digestion of pON-CMVenv generated a 4.8-kilobase fragment containing the entire CMV IE promoter, the FeLV *env* gene, and the simian virus 40 polyadenylation and enhancer regions. This fragment was adapted, using *Hind*III linkers, and inserted into the *Hind*III site of pGC113. The resulting plasmid, pGC113-*env* (pGC114), served as an insertion vector to introduce the CMV IE-FeLV *env* expression cassette into the FHV genome via homologous recombination. In pGC114, the expression cassette is oriented in the same direction as the FHV TK gene. Similar manipulations were performed to derive insertion vectors based on pGC111. Plasmids containing the expression cassette in both orientations were obtained (pGC112 and pGC112/flip).

PCR was used to adapt the FeLV *gag* and protease genes of the pFGA-5 provirus for molecular cloning into expression cassettes. PCR primers were designed, using the nucleotide sequence of another isolate of FeLV subgroup A, FeLV-F6A (11). The following 34-mer was used to prime PCR at the 5' end of the *gag* gene: 5'GCTCTAGACCCGGGACCACCGACCCACCATCAGG3'. This primer includes nucleotides 517 through 542 of FeLV-F6A and a 5' extension containing an *Xba*I site to permit molecular cloning at the *Xba*I site of pON1-CMVIE. The oligonucleotide used to prime PCR at the 3' end of the protease gene was as follows: 5'CCGATATCTTATCAAAGGACTTGTAGGGGTAAAC3'. Features of the complement of this 34-mer include nucleotides 2767 through 2786 at the carboxy terminus of the protease gene, tandem TGA and TAA termination codons, and an extension containing an *EcoRV* site to enable molec-

ular cloning in pON1-CMVIE (at the *Pvu*II site). Colony hybridization was used to identify the desired plasmid (pFeLVgag3).

The *gag* + protease fragment was transferred into pON1-CMVIE, replacing the  $\beta$ -galactosidase gene, to generate pON-CMVgag. The *EcoRI* fragment containing the expression cassette was then inserted into the *Hind*III site of pGC113. The resulting plasmid (pGC113-*gag*; pJN116) contained the cassette oriented in parallel with FHV TK.

**DNA transfection methods and recombinant virus isolation.** DNA transfection utilized the calcium phosphate precipitation method (21). The promoter strength of herpesvirus promoters in FHV-infected cells was evaluated by the transient expression of  $\beta$ -galactosidase from pON1-based plasmids (64). Qualitative judgments were obtained by histochemical staining for  $\beta$ -galactosidase activity (60). Quantitation of  $\beta$ -galactosidase-specific enzymatic activity was as described previously (64).

Recombinant FHVs were obtained as described by Nunberg et al. (50). Progeny viruses resistant to thymidine arabinoside (Raylo Chemicals, Edmonton, Alberta, Canada) were screened for expression of FeLV antigens by immunostaining of viral plaques. Expressing viruses were purified to homogeneity by two additional cycles of plaque purification and immunostaining.

**Protein and immunologic analyses.** Cells were fixed for immunohistochemical analysis by using -20°C methanol. FeLV gp70 was visualized by using monoclonal antibody (McAb) 25.5 (41) which was kindly provided by Niels Pedersen (University of California School of Veterinary Medicine, Davis, Calif.). Immunostaining was done with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin G antibody (Accurate Chemicals, Westbury, N.Y.) and 3,3'-diaminobenzidine (Sigma Chemical Co., St. Louis, Mo.) as substrate. Immunofluorescence was obtained by using fluorescein-labeled goat anti-mouse immunoglobulin G antibody (Fab) (Jackson ImmunoResearch Laboratories, West Grove, Pa.). Surface immunofluorescence experiments used intact cells fixed with 2% formaldehyde-0.2% glutaraldehyde. FeLV *gag* expression was determined by protein blot analysis (20) of whole cell extracts using the FeLV p27-specific McAb MC-1 provided by Niels Pedersen (42). Blots were developed using horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin G antibody and enhanced chemiluminescence detection (Amersham Corporation, Arlington Heights, Ill.).

Radioimmunoprecipitation studies were performed using CRFK cells that had been metabolically labeled for 18 h using [<sup>35</sup>S]methionine (20). FeLV gp70 was detected using McAb C11D8 (22) which was kindly provided by Chris Grant (Pacific NorthWest Research Foundation, Seattle, Wash.). Immunoprecipitation from the cell surface entailed incubation of intact cell monolayers with McAb C11D8 in serum-free medium containing 1% bovine serum albumin-5  $\mu$ g of aprotinin per ml. Immune complexes were formed at 4°C for 90 min, and monolayers were carefully washed before lysis and immunoprecipitation.

Endoglycosidases F and H (Endo F and H, respectively) were obtained from Boehringer Mannheim. The Endo F preparation contains glycopeptidase F which cleaves the asparaginyl-oligosaccharide linkage to completely remove N-linked glycosylation. Radiolabeled immune complexes were stripped from protein-A Sepharose by boiling the mixture in 0.2% sodium dodecyl sulfate (SDS)-1.5% 2-mercaptoethanol. Digestion mixtures were as prescribed by the supplier.

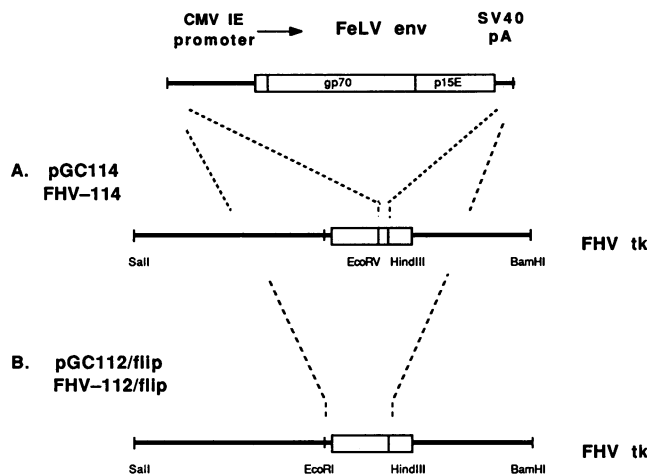


FIG. 1. Schematic representation of recombinant FHV plasmids and viruses. The expression cassette comprising the CMV IE promoter, the FeLV-A/FGA *env* gene, and the pON1-derived simian virus 40 polyadenylation site and enhancer was inserted into the FHV TK gene by using either *EcoRV* and *HindIII* sites within the TK coding region (pGC114; FHV-114) (A) or an *EcoRI* site upstream of the TK coding region and the internal *HindIII* site (pGC112/flip; FHV-112/flip) (B). The starting TK deletion plasmids (pGC113 and pGC111, respectively) contain the TK gene and flanking sequences as a *Sall*-*BamHI* fragment (5.5 kilobases in the parental FHV UT88).

## RESULTS

### Development of expression cassette for recombinant FHV.

We previously described the construction of a recombinant TK<sup>-</sup> FHV, FHV-113, bearing an *EcoRV* to *HindIII* deletion in the coding sequence of the FHV TK gene (50). To develop expression cassettes to drive expression of heterologous genes in recombinant FHV, we explored the use of the major CMV IE promoter (3, 65). This promoter has been shown to function in a variety of heterologous environments, including recombinant herpesviruses (16, 64). pON1-CMVIE, containing the CMV IE promoter upstream of a modified *E. coli*  $\beta$ -galactosidase gene, was introduced into feline CRFK cells by calcium phosphate-mediated transfection, and transient expression of  $\beta$ -galactosidase was determined subsequent to FHV infection. High levels of CMV IE promoter expression were obtained in FHV-infected cells. In comparison, the  $\alpha 4$  promoter of herpes simplex virus type 1 (*PvuII*-*BamHI* of pRB403; 43) was substantially less active (data not shown). Expression of the CMV IE promoter was reduced approximately threefold in the absence of concurrent FHV infection, suggesting the ability of FHV to transactivate the CMV IE promoter (56). Thus, the CMV IE promoter appeared well suited for use in expression cassettes for recombinant FHV.

**Recombinant FHV expressing FeLV *env*.** The  $\beta$ -galactosidase gene of pON1-CMVIE was replaced by the *env* gene of FeLV subgroup A, and the CMV IE-FeLV *env* expression cassette was then transferred into *ptk* $\Delta$ *EcoRV*-*HindIII* (pGC113) (Fig. 1A). pGC113 had previously been used to derive the recombinant TK<sup>-</sup> virus FHV-113 (50). The final FeLV *env* insertion vector was designated pGC113-*env* (pGC114). The FeLV *env* expression cassette was also inserted into another TK deletion plasmid, *ptk* $\Delta$ *EcoRI*-*HindIII* (pGC111), which contains a larger deletion spanning the presumed 5' untranslated region of TK (Fig. 1B).

pGC111-*env*/flip (pGC112/flip) contained the cassette oriented in the same 3'→5' direction as the TK gene.

Recombinant FHV containing these expression cassettes were derived by using standard methods of marker rescue (50, 63). All thymidine arabinoside-resistant (araT<sup>r</sup>) plaques derived using pGC114 contained FeLV *env*-expressing virus. An example of the immunostaining of one such isolate (designated FHV-114) is shown in Fig. 2A. The genetic structure of FHV-114 was confirmed by DNA blot analysis; the cassette was inserted without rearrangement at the expected site within the FHV TK gene (data not shown).

FHV-114 grew well in culture. Virus yields ( $10^8$  to  $10^9$  PFU/ml) and plaque morphology were comparable to those of the parental FHV UT88 and the recombinant TK<sup>-</sup> virus FHV-113. Expression of FeLV *env* was stable on large-scale growth of the virus. In contrast to the ease with which FHV-114 was isolated, efforts to isolate pGC112-derived viruses were hampered by the low initial yield of FeLV *env*-expressing araT<sup>r</sup> plaques and by the slow development of these plaques. Once purified, both FHV-112 and FHV-112/flip were stable and expressed FeLV *env* protein (Fig. 2B). Both viruses grew poorly, attaining titers of  $10^6$  to  $10^7$  PFU/ml after 5 to 6 days. Plaques were small and syncytial in appearance. We speculate that the phenotype derives from the deletion of sequences at the 5' end of TK, sequences which encode the FHV homolog of herpes simplex virus type 1 UL24 (50). Jacobson et al. (30) had previously reported that herpes simplex virus type 1 mutants in UL24 are substantially impaired for growth and display phenotypes similar to those noted here.

**Expression of FeLV *env* protein.** Expression of the FeLV *env* protein in cells infected with FHV-114 was examined by radioimmunoprecipitation (Fig. 3A). Analysis of cells persistently infected with authentic FeLV-A/FGA (lane c) revealed two proteins, the gp85 *env* precursor and the mature gp70. Only gp70 was found in FeLV virions (lane d). Cells infected with the recombinant FHV-114 (lane b) contained a protein that comigrated with authentic FeLV gp85. In addition, these cells expressed a heterogeneous smear of immunoreactive proteins ranging in apparent molecular size from 65 to 95 kilodaltons (kDa). In most experiments, one could also discern within this smear an underlying diffuse band of gp70. None of these proteins was found in cells infected with the parental FHV UT88 (lane a). (A 45-kDa glycoprotein was often observed in samples derived from FHV-114-infected cells; we presume this to be a degradation product of gp85.)

To confirm the protein assignments, we deglycosylated the immunoprecipitated proteins to determine the polypeptide molecular sizes (Fig. 3B). Treatment of gp70 from FeLV virions with Endo F-glycopeptidase F yielded a protein with an apparent molecular size of 47 kDa (lane d), consistent with that of the polypeptide backbone of gp70. FeLV-infected cells gave rise to an additional protein, a 67-kDa polypeptide derived from the gp85 precursor (lane c). Deglycosylation of proteins from cells infected with FHV-114 (lane b) resulted in generation of the same two bands, consistent with the assignment as gp85 and gp70.

We concluded that the FeLV *env* protein in FHV-114-infected cells accumulated largely as the gp85 precursor protein. Proteolytic processing to generate the mature gp70 protein occurred in FHV-114 infection, but to a limited extent compared with that in cells infected with authentic FeLV. Although the CMV IE promoter is an early promoter (31), FeLV *env* protein continued to accumulate throughout late stages of FHV infection (data not shown; E. A. Petrovskis and L. E. Post, personal communication).

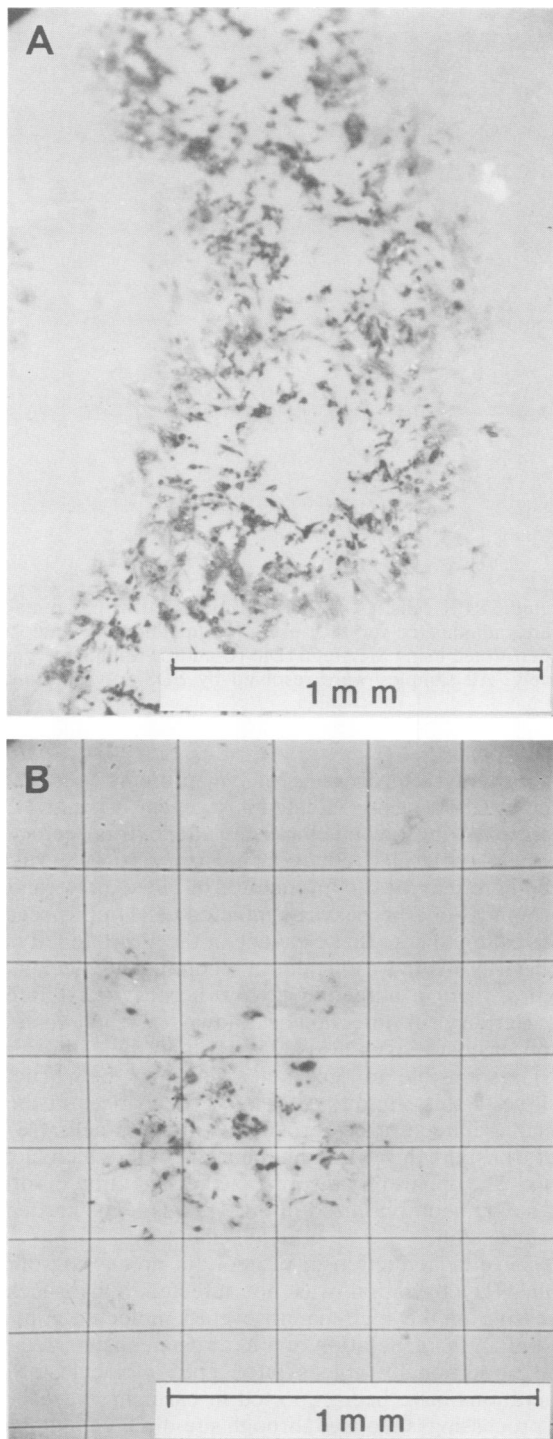


FIG. 2. Expression of FeLV *env* in FHV-114 and FHV-112 plaques. CRFK cells were infected with FHV-114 (A) or FHV-112 (B) and fixed, using cold methanol when viral plaques were evident (3 and 6 days postinfection, respectively). Indirect immunostaining was performed using McAb 25.5 and horseradish peroxidase-3,3'-diaminobenzidine detection. Plaques derived from parental FHV UT88 or from FHV-113 did not stain with these procedures.

Proteolytic cleavage of retroviral *env* proteins occurs concomitantly with glycosylation and transport through the Golgi apparatus (14). We were interested to determine whether glycosylation was affected in FHV-114-infected cells. Endo H digestion was used to determine the degree of complex glycosylation (Fig. 3C). As expected, FeLV gp85 was sensitive to Endo H treatment (lane c); the reduction in apparent molecular size to that of the fully deglycosylated protein indicated that only high-mannose core structures were present. In contrast, mature gp70 was largely resistant to Endo H treatment, showing only a slight reduction in apparent molecular size upon treatment (lane d). A fully deglycosylated polypeptide of 47 kDa was not observed, indicating that mature FeLV gp70 contained predominantly hybrid and complex glycosylation. Likewise, in cells infected with FHV-114, gp85 appeared to be sensitive to Endo H treatment and gp70 appeared to be resistant (lane b). The heterodisperse proteins of 65 to 95 kDa were also resistant to digestion. We speculate that these Endo H-resistant glycoproteins represent forms of gp85 in which terminal modification of the N-linked oligosaccharide had occurred. In the absence of efficient proteolytic processing, oligosaccharide modification continued and a population of terminally glycosylated gp85 proteins was generated.

To explore intracellular transport and cell surface localization of the FeLV *env* proteins, intact FHV-114-infected cells were stained, using McAb 25.5 (Fig. 4A). Despite inefficient proteolytic processing, FeLV *env* protein was clearly expressed on the surfaces of FHV-114-infected cells. Circumferential staining consistent with surface localization was typically observed. Intracellular localization was also examined (Fig. 4B). Although in some cells the fluorescence was predominantly perinuclear and presumably Golgi associated, the majority of cells showed a less-organized cytoplasmic staining pattern.

To examine further the cell surface expression of the *env* protein, we immunoprecipitated proteins from the surfaces of live cells (Fig. 5B). Approximately 10 to 15% of the total *env* protein in FHV-114-infected cells was available at the surface (compare lanes b of panels A and B). This proportion was somewhat less than that seen in FeLV-infected cells, in which approximately 30 to 40% of total *env* protein was at the surface (lanes c). It was interesting to note that the molecular forms of the *env* protein expressed on the surfaces of cells infected with either virus reflected those found intracellularly (compare panels A and B).

**Recombinant FHV expressing FeLV *gag* plus protease.** We were also interested to explore the role of internal *gag* immunogens in protective immunity. We used PCR to adapt the FeLV *gag* gene and the contiguous *pol*-encoded protease for expression in CMV IE promoter expression vectors. The entire pON-CMVgag expression cassette was transferred into pGC113, and one such plasmid, pGC113-gag (pJN116), was used to generate recombinant FHVs. As with pGC113-based viruses expressing *env*, FHV-116 produced clean plaques within 2 to 3 days, was stable through plaque purification, and yielded  $10^8$  to  $10^9$  PFU/ml on subsequent growth.

**Expression of FeLV *gag* plus protease protein.** To determine whether the *gag*-protease precursor protein was expressed by FHV-116 and whether this protein was able to effect proteolytic processing of the FeLV *gag* precursor (6, 38, 47), we subjected whole-cell extracts of FHV-116-infected CRFK cells to protein blot analysis, using McAb MC-1 (Fig. 6). The predominant *gag*-related protein expressed by FHV-116 (lane a) comigrated with Pr65<sup>gag</sup> from

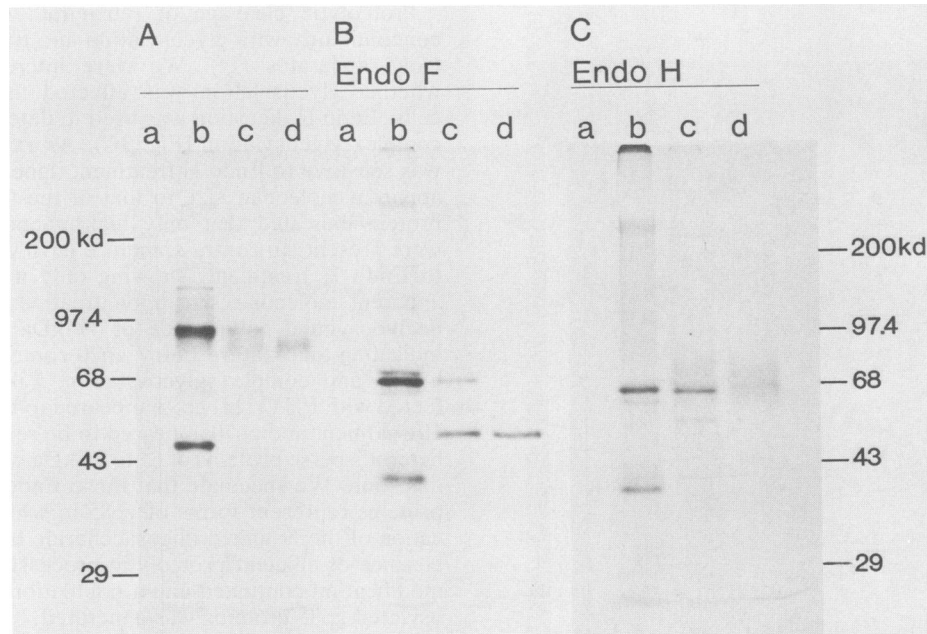


FIG. 3. Characterization of FeLV *env* protein expressed in FHV-114-infected CRFK cells. (A) CRFK cells infected with parental FHV UT88 (lane a), recombinant FHV-114 (lane b), or FeLV-A/FGA (lane c) were radiolabeled for 18 h using [<sup>35</sup>S]methionine. Radiolabeled FeLV-A/FGA virions were also prepared (lane d). Immunoprecipitation was performed using McAb C11D8. (B and C) Immunoprecipitated proteins were treated with Endo F-glycopeptidase F or Endo H, respectively. All samples were resolved by SDS-polyacrylamide gel electrophoresis. Molecular size markers are indicated.

cells persistently infected with authentic FeLV (lane b). The *gag*-protease precursor protein was not expected to accumulate to high levels and was not identified in these experiments. However, functional protease was clearly produced in cells infected with FHV-116; specific proteolytic processing products of Pr65<sup>gag</sup> were readily observed. The mature p27 in FHV-116-infected cells comigrated with that of purified FeLV virions (lane v). Processing intermediates were also similar to those seen in FeLV-infected cells. The overall extent of Pr65<sup>gag</sup> processing was comparable in FHV-116 and in FeLV infection.

#### DISCUSSION

Recombinant viruses expressing heterologous immunogens may provide useful reagents with which to dissect the immune response to infection. Such live-virus vectors can be used to present specific immunogens to both humoral and cellular arms of the host immune system, as in natural infection, but isolated from the pathogenic effects of that infection. To explore the bases for retroviral immunity in the cat, a species susceptible to both FeLV oncornavirus and feline immunodeficiency virus lentivirus infection, we chose to develop live recombinant virus vectors based on the feline-specific herpesvirus FHV. In our previous work, we demonstrated that recombinant FHV-defective in TK could be isolated. Here we describe several recombinant FHV-defective in TK in which FeLV *env* and *gag* gene expression cassettes have been inserted within the FHV TK gene. Expression utilized the strong CMV IE promoter.

The FeLV *env* protein expressed by FHV-114 is processed and transported to the surfaces of infected cells as in authentic FeLV infection, with the exception that proteolytic cleavage of the gp85 precursor protein in FHV-114 infection is less efficient than that in FeLV infection. Effi-

cient proteolytic processing of *env* proteins has been observed in studies using isolated *env* genes (71), as well as in studies involving vaccinia virus and adenovirus vectors (4, 8, 13, 20, 28, 34). The reduction in proteolytic cleavage observed here may be a consequence of *env* expression in the environment of a herpesvirus-infected cell. In this regard, it is interesting to note that cleavage of the gI protein of bovine herpesvirus 1 is more efficient in recombinant vaccinia virus infection than in authentic bovine herpesvirus 1 infection (69). Herpesvirus infection is known to result in a rapid shutoff of host biosynthesis (15, 51), and the cellular protease(s) responsible for gp85 cleavage may be particularly sensitive to this shutoff. Alternatively, changes in the subcellular architecture of herpesvirus-infected cells (48) may affect transport through the compartment in which cleavage occurs. The apparently disorganized distribution of intracellular *env* antigen observed in our studies lends credence to this suggestion.

Despite the reduction in proteolytic processing of gp85, terminal glycosylation was not affected. Oligosaccharide processing of the high-mannose gp85 molecule continues, resulting in the generation of a heterogeneously sized population of terminally glycosylated gp85 molecules. Similar observations have been reported in cases in which proteolytic processing is blocked through site-directed mutagenesis to the *env* cleavage site (17, 18, 46, 54). As has also been noted by others, transport to the cell surface requires neither terminal glycosylation nor proteolytic cleavage (9, 17, 18, 46, 52, 54). All forms of the FeLV *env* protein are efficiently transported to the surfaces of FHV-114-infected cells.

Expression of the FeLV *gag* + protease gene appears to be unperturbed in FHV-116-infected cells. Suppression of the endogenous FeLV *gag* termination codon to allow synthesis of the *gag*-protease precursor protein occurs, and



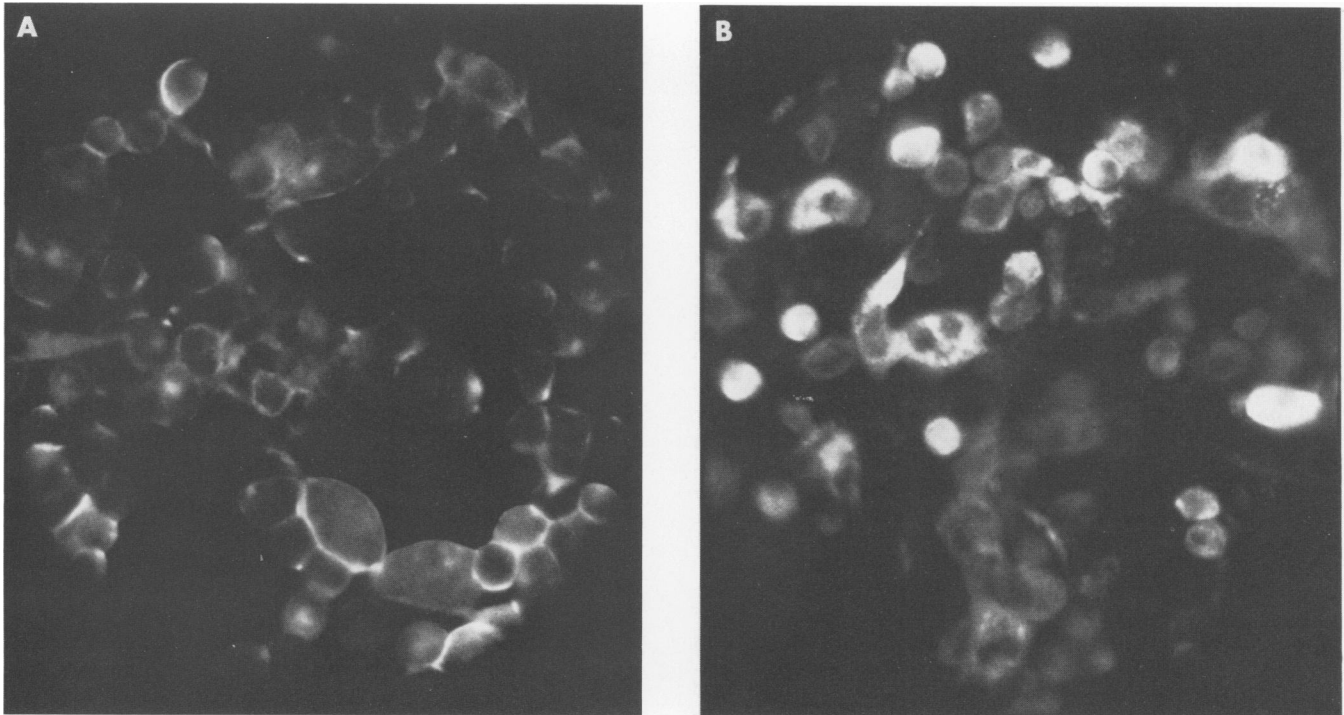


FIG. 4. Indirect immunofluorescence of CRFK cells infected with FHV-114. CRFK cells were infected with FHV-114 and fixed for immunofluorescence at 2 days postinfection by using either formaldehyde-glutaraldehyde (A) or cold methanol (B). Cells fixed using the former method are intact, and only surface proteins are available to antibody. FeLV *env* expression was detected using McAb 25.5. Staining was observed by using a Zeiss epifluorescence microscopy at 1,000 $\times$  magnification and was photographed on automatic exposure setting, using Kodak Ektachrome ASA 800 film. Exposure times to detect surface fluorescence were substantially longer than those necessary to detect whole-cell fluorescence. Cells infected with parental FHV UT88 did not show specific fluorescence.

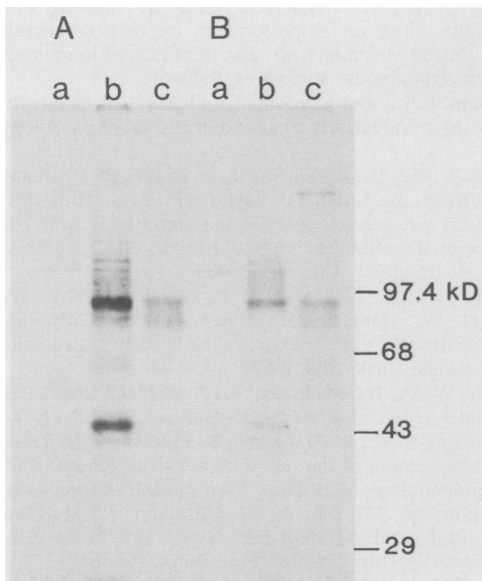


FIG. 5. Immunoprecipitation of FeLV *env* from the surfaces of CRFK cells infected with FHV-114. CRFK cells infected with parental FHV UT88 (lanes a), recombinant FHV-114 (lanes b), or FeLV-A/FGA (lanes c) were radiolabeled as previously described. (A) Cells were lysed before immunoprecipitation, using McAb C11D8 (total cell protein). (B) Antibody was incubated with intact cell monolayers before lysis and immunoprecipitation (surface protein). Samples were resolved by SDS-polyacrylamide gel electrophoresis. Molecular size markers are indicated.

the resulting protein yields functional protease which mediates proteolytic maturation of the Pr65<sup>gag</sup> precursor. Several groups have further shown that retroviral *gag* proteins are capable of self-assembly and budding (19, 29, 33, 61). In subsequent work, we will examine the assembly and budding of FeLV *gag*-containing particles in FHV-116-infected cells.

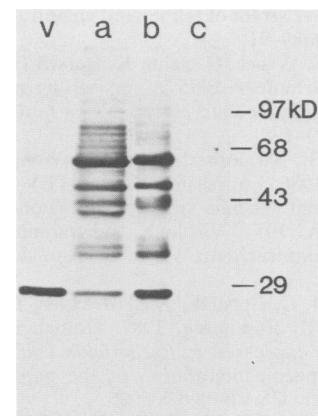


FIG. 6. Protein blot analysis of FeLV *gag* + protease expression in CRFK cells infected with FHV-116. Whole-cell extracts were prepared of CRFK cells infected with recombinant FHV-116 (lane a), FeLV-A/FGA (lane b), or parental FHV UT88 (lane c). Proteins (40  $\mu$ g each) were resolved by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane. Immunodetection was done by using McAb MC-1 and ECL. Partially purified FeLV-A/FGA virions (6  $\mu$ g) (lane v) provided a molecular size control for authentic FeLV p27. Molecular size markers are indicated.

One ultimate goal of this work is to develop recombinant FHV for use as vaccine vectors. Studies to assess the safety and immunogenicity of these recombinant FHV are planned. Attenuation of the virus for safe use in cats may be provided by the defect in TK (12, 35, 36, 45, 67). Preliminary studies in cats, using FHV-114, indicate that the FeLV *env* protein expressed during vaccinal infection is immunogenic (R. C. Wardley, P. J. Berlinski, and A. L. Meyer, personal communication). The current use of conventional attenuated FHV vaccines speaks to the safety and efficacy of this feline-specific virus in routine vaccination. Building on this, we propose that recombinant FHV may serve as versatile vectors for the expression of protective immunogens in the vaccination of cats.

The use of recombinant FHV vectors to present FeLV *env* and *gag* immunogens in vaccinal infection may further provide insight as to the role of these retroviral proteins in protective immunity. These studies, as well as those involving the feline lentivirus feline immunodeficiency virus may help to elucidate immunologic mechanisms critical for protection against human retroviral disease.

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