Strong Sequence Conservation among Horizontally Transmissible, Minimally Pathogenic Feline Leukemia Viruses

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We report the first complete nucleotide sequence (8,440 base pairs) of a biologically active feline leukemia virus (FeLV), designated FeLV-61E (or F6A), and the molecular cloning, biological activity, and *env*-long terminal repeat (LTR) sequence of another FeLV isolate, FeLV-3281 (or F3A). F6A corresponds to the non-disease-specific common-form component of the immunodeficiency disease-inducing strain of FeLV, FeLV-FAIDS, and was isolated from tissue DNA of a cat following experimental transmission of naturally occurring feline acquired immunodeficiency syndrome. F3A clones were derived from a subgroup-A-virus-producing feline tumor cell line. Both are unusual relative to other molecularly cloned FeLVs studied to date in their ability to induce viremia in weanling (8-week-old) cats and in their failure to induce acute disease. The F6A provirus is organized into 5'-LTR-*gag-pol-env*-LTR-3' regions; the *gag* and *pol* open reading frames are separated by an amber codon, and *env* is in a different reading frame. The deduced extracellular glycoproteins of F6A, F3A, and the Glasgow-1 subgroup A isolate of FeLV (M. Stewart, M. Warnock, A. Wheeler, N. Wilkie, J. Mullins, D. Onions, and J. Neil, *J. Virol.* 58:825-834, 1986) are 98% homologous, despite having been isolated from naturally infected cats 6 to 13 years apart and from widely different geographic locations. As a group, their envelope gene sequences differ markedly from those of the disease-associated subgroup B and acutely pathogenic subgroup C viruses. Thus, F6A and F3A correspond to members of a highly conserved family and represent prototypes of the horizontally transmitted, minimally pathogenic FeLV present in all naturally occurring infections.

Feline leukemia viruses (FeLV) are exogenous type C retroviruses that are responsible for induction of a diverse series of lymphoreticular diseases of outbred cats including lymphosarcoma, leukemia, aplastic anemia, myelodysplasia, and feline acquired immunodeficiency syndrome (20, 25, 27). FeLV have been classified into subgroups A, B, and C on the basis of virus interference and neutralization assays (31, 55, 56), and their distribution differs widely. Subgroup A viruses have been found in all naturally occurring infections examined (29). Subgroup B, found in approximately 40% of all infections, and subgroup C, found in perhaps 1% of infections, occur as mixed infections of subgroups A and B, A and C, or A, B, and C (29). Subgroup identity also correlates with the host range of infection in vitro and pathogenicity in cats: FeLV-A isolates are sometimes restricted to growth in feline cells and are minimally pathogenic; FeLV-B isolates grow in heterologous cells such as human and canine fibroblastoid cells and are found at a higher frequency in cats with proliferative disease; and the rare FeLV-C isolates have an extended host range including human, canine, and guinea pig cells and are capable of inducing aplastic anemia (23, 28-31, 51). The viral determinants of FeLV subgroup specificity are not yet known, but probably correspond to regions of the extracellular glycoprotein (gp70) (62), as has been shown for avian retroviruses (9).

Studies with molecularly cloned FeLVs have extended earlier experimental transmission studies and clearly indicate that FeLV genomes have distinctly different biological

properties. For example, molecularly cloned FeLV-C-Sarma (FSC) induces aplastic anemia in 100% of inoculated, viremic, outbred domestic cats within 2 months of inoculation (51), whereas cloned FeLV-B-Gardner-Arnstein (FGB) has been shown to induce myeloproliferative syndrome, thymic lymphoma, and fibrosarcoma in some inoculated cats 2 to 12 months postinoculation (E. A. Hoover and J. I. Mullins, unpublished results).

The FeLV-FAIDS virus strain, a subgroup A and B mixture, induces lethal immunosuppressive disease in nearly 100% of newborn through adult viremic cats (25, 26) and has provided another source of molecularly cloned viruses with distinct biological properties. Two major forms of FeLV DNA, distinguishable by restriction site differences, are present in FeLV-FAIDS-infected cats (43; J. I. Mullins, S. L. Quackenbush, and E. A. Hoover, manuscript in preparation). Common-form virus is detected at the onset of viremia and persists throughout the lifetime of the animal. In contrast, the appearance of variant viral genomes, recognizable by restriction site polymorphisms in the *env* region, precedes and predicts the onset of immunodeficiency syndrome. Variant viruses replicate preferentially in tissues such as bone marrow and intestine and persist in high copy number as unintegrated viral DNA.

The molecularly cloned provirus FeLV-61E encodes a replication-competent virus corresponding to the FeLV-FAIDS common-form genome. Nearly all weanling-age cats inoculated with 61E-derived virus (F6A) become viremic within 2 to 4 weeks. None of 12 F6A-inoculated cats developed immunodeficiency disease 1 year or longer after inoculation, and only 1 of 4 has developed disease within 2 years: a T-cell lymphosarcoma detected 20 months after

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TABLE 1. Biological activity of molecularly cloned F6A and F3A proviruses

Virus	RT activity (cpm/reaction) ^a	In vivo analysis of cats ^b			
		Age at inoculation	No. viremic/ no. inoculated	Disease produced ^d	Survival (weeks)
F6A	29,000	4 days 2-3 mo	2/2 9/10	None None (8) lymphoma (1)	>87 >66-108 80
F3A-2	20,400	2 days 56 days	4/4 3/3	None None	>24 >24
F3A-50	149	ND ^c			
5'F3A-50-3'FGB	186	ND			
5'FGB-3'F3A-50	166,650	ND			

^a Reverse transcriptase (RT) activity in cell-free supernatants collected from feline embryo fibroblast cultures was determined 21 to 36 days after transfection. Each cpm value represents the average of two experiments to determine counts of tritiated TTP incorporated into acid-insoluble DNA (45). Negative control values ranged up to 890 cpm.

^b Weanling-age specific-pathogen-free cats were inoculated intraperitoneally, and viremia and signs of clinical disease were monitored thereafter as described in Materials and Methods.

^c Viremia was detected between 2 and 3 weeks postinoculation.

^d Numbers in parentheses refer to the number of cats with the disease.

^e ND, Not done.

inoculation (Table 1; J. Overbaugh, P. R. Donahue, E. A. Hoover, S. L. Quackenbush, and J. I. Mullins, submitted for publication). F6A is therefore considered to be minimally pathogenic. By contrast, all FeLV-FAIDS variant clones studied to date are replication defective in vitro. However, when rescued as transmissible virus by F6A, variant-common-form virus mixtures induce fatal immunodeficiency disease in vivo (Overbaugh et al., submitted).

The genetic differences which underlie the unique pathogenicity of different FeLV genomes can be identified through nucleotide sequence analysis. Such analyses have to date been confined to the envelope and long terminal repeat (LTR) (10, 11, 17-19, 38, 46, 51, 59, 65) and, in one case of a defective virus, to the *gag* region (35). As a first step in evaluating the nucleic acid differences between pathogenic and minimally pathogenic viruses and to elucidate the overall genetic structure of FeLV, we determined the complete 8,440-base-pair (bp) nucleotide sequence of the biologically characterized FeLV F6A. We also molecularly cloned a distinct subgroup A FeLV, from the 3281 tumor cell line (FeLV-3281-A or F3A), determined the nucleotide sequence of its envelope and LTR region, and studied its biological activity. Comparison of envelope gene sequences reveals that F6A represents a highly conserved prototype of the horizontally transmitted and minimally pathogenic virus found in possibly all naturally occurring FeLV infections.

MATERIALS AND METHODS

Molecular cloning and DNA sequencing. FeLV cross-reactive proviruses were molecularly cloned from the 3281 tumor cell line which produces only F3A (15). The tumor was obtained from a pet cat in New York City in 1977 (E. Zuckerman and W. D. Hardy, Jr., personal communication). Procedures used for cloning in bacteriophage Charon 4A, screening, and analysis of clones have been described previously (41, 42). The molecular cloning, restriction site mapping, and biological activity of F6A have been described (Overbaugh et al., submitted).

The F6A provirus was cloned directly from intestinal tissue DNA of a specific-pathogen-free cat following inoculation with lymphosarcoma cell-free supernatant from a pet cat from Fort Collins, Colo., in 1983 (26). An *EcoRI* frag-

ment containing the full-length F6A provirus and host flanking sequences was subcloned into pUC18. Deletion clones were then generated by digestion with exonuclease BAL31 (49), ligated into M13mp18, and sequenced by the dideoxy chain termination method (54). A 2.5-kilobase-pair *PstI* fragment from the 3' end of F3A (clone 50) was subcloned into M13mp18, and additional M13 subclones were generated by using *HindIII* and *BalI* sites located within the *PstI* fragment. These overlapping fragments were sequenced as described above.

The IntelliGenetics program GEL was used for managing the sequencing project, and IFIND, based on the algorithm of Wilbur and Lipman (63), and XFASTP (37) were used for nucleotide and amino acid sequence alignments.

Biological analysis of F3A clones. F3A clones 2 and 50 were transfected into the AH927 feline embryo fibroblast cell line and monitored for the production of extracellular reverse transcriptase (45). A total of 19 specific-pathogen-free cats, 6 newborns and 13 2- to 3-month-old (weanling) cats from a breeding colony maintained in the Department of Pathology, Colorado State University, were inoculated with 5×10^4 to 1×10^5 focus-forming units (13, 52) of transfection-derived F3A clone 2 and F6A (Table 1). Following inoculation, serial weekly, then biweekly, and then monthly determinations were made of clinical symptoms, body weight, circulating blood cell numbers and morphology, bone marrow colony-forming progenitor cells (1, 14), and the presence of FeLV p27 antigen in circulating leukocytes and platelets (21, 24) to assess the presence of FeLV infection, the establishment of viremia, and the occurrence of hematologic or other disease.

Construction of F3A virus chimeras. Chimeric viruses were constructed by exchanging viral fragments around the conserved *XhoI* site at the border of the *pol* and *env* genes (position 5818 of F6A). The recombinant 5'FGB-3'F3A-50 was constructed by ligating the 5' 1.2-kilobase *EcoRI-XhoI* fragment from pFGB (41) into the *EcoRI-XhoI* sites of a 3' *EcoRI-BamHI* pUC19 subclone of pF3A-50. This construct was then digested with *XhoI* and ligated with the gel-purified 5.2-kilobase internal fragment of pFGB to generate a full-length virus. To construct the 5'F3A-50-3'FGB chimera, an *EcoRI-HindIII* subclone (in pUC19) of the 3' region of pFGB was digested with *EcoRI* and *XhoI* and then ligated with a gel-purified *EcoRI-XhoI* fragment representing the 5' portion of F3A-50.

FIG. 1. Complete nucleotide sequence of F6A and the envelope and 3'-LTR sequence of F3A. The F3A sequence begins at position 5920 in F6A; F3A nucleotide substitutions are designated above the F6A sequence. Deduced F6A amino acid sequences are designated by the one-letter code below the DNA sequence, and F3A substitutions are noted in three-letter code above the sequence. Abbreviations: PBS, primer-binding site; SD, splice donor; SA, splice acceptor. Z is the translated sequence at position 2424 corresponding to an amber stop codon. This codon is likely to be read as a glutamine residue in the translation of the protease protein of *pol* (66).

FeLV genome organization. The complete 8,440-bp sequence of the F6A provirus, its predicted protein products, and the 2,521-bp envelope–3′-LTR sequence of F3A (clone 50) are shown in Fig. 1. Open reading frames (ORFs) corresponding to *gag* (encoding the nucleocapsid proteins), *pol* (encoding protease, reverse transcriptase, and endonu-

clease-integrase), and *env* (encoding the extracellular and transmembrane envelope proteins) within the F6A and F3A sequences are shown in Fig. 2. In F6A, a 1,791-bp *gag* ORF and a 3,609-bp *pol* ORF are in frame and separated by a single amber stop codon. The 2,271-bp *env* ORF is found in a separate translational reading frame which overlaps the 3'-terminal 310 bp of *pol*. Conceptual translation of the ORFs shown in Fig. 1 begins at methionine 1 of *gag* (position

FIG. 2. ORFs of F6A and F3A. Frames 1 to 3 of each coding strand are represented. Vertical bars mark positions of termination codons. Boxes represent the ORFs (*gag*, *pol*, and *env*) known to encode viral proteins and the positions of the LTRs. Asterisks mark the positions of the likely initiating methionines for the *gag* (*gag-pol*) and *env* translation products.

include consensus Goldberg-Hogness and CCAAT regulatory sequences and a consensus polyadenylation recognition signal.

The F6A LTR shares 98% homology with that of F3A and FeLV-A-Glasgow-1 (FGA) (59), 94% homology with the FSC LTR (51) and 97% homology with the FGB LTR (10), aside from 1-bp gaps (except for the FSC LTR, which also has a 6-bp insertion) introduced for maximum alignment.

An 18-bp proline-tRNA binding site begins at position 485 in F6A, and a consensus splice donor site (40) homologous to the known murine splice donor (36) used for generation of the subgenomic *env* mRNA is located at position 542. The 201-bp interval between the 5'-LTR and methionine 1 of *gag* is also conserved, showing greater than 95% homology with FSB (35) and two feline sarcoma viruses (GA-FeSV and ST-FeSV) (19).

F6A *gag* gene. Two in-frame initiation codons (positions 684 and 906) occur near the 5' end of the F6A *gag* ORF. The intervening deduced primary sequence is conserved in 71 of 74 positions when compared with FSB and contains a signal sequence which probably directs the production of glycosylated *gag* proteins (35) known to be secreted by FeLV-infected cells (44) including F6A-infected feline fibroblasts (M. Poss, personal communication). Two consensus mRNA splice acceptor sites (positions 748 and 897) (40) occur between *gag* methionines 1 and 2, thus potentiating the removal of methionine 1 by splicing, although no studies to date have addressed this possibility. GA-FeSV and SM-FeSV each contain a 12-base (4-amino-acid) insertion in this region (18, 19) which is not present in other reported FeLV and FeSV sequences, including F6A (Fig. 3).

The F6A *gag* ORF encodes a 501-amino-acid (57-kilodalton [kDa]) protein that probably corresponds to the nonglycosylated precursor polyprotein Pr65^{gag}, which is predicted to begin at *gag* methionine 2 (19). Pr65^{gag} contains, in order, the p15, p12, p27, and p10 virion core structural peptides generated by proteolytic cleavage (34). The reported primary sequence of p10 places the carboxy terminus of Pr65^{gag} 12 bp upstream of the amber stop codon in *pol* (7). Internal *gag* peptide junctions may be assigned from the known amino-terminal primary sequences of FeLV

p27 and p10 (7, 47) and alignment of p15 and p12 sequences with amino- and carboxy-terminal sequences of murine leukemia virus (MuLV) *gag* products (48). The predicted Pr65^{gag} proteins of F6A and FSB are 98% homologous, and the reported primary sequence of p10 and deduced p10 proteins of FSB and F6A differ only at residue 15 (Fig. 3) (7, 35).

F6A *pol* gene. Previous analyses of retroviral *pol* ORFs predicted that the corresponding ORF in F6A should encode three mature peptides: protease, reverse transcriptase, and endonuclease-integrase (8, 33). The N-terminal primary sequence of an FeLV protease has been determined (65) and is homologous at 13 of 15 deduced residues in F6A. This homology begins at position 2412, immediately adjacent to the C terminus of *gag* p10. The glutamine residue at amino acid 5 of protease would originate from tRNA suppression of the stop codon at the beginning of the *pol* ORF. These findings, together with the lack of a methionine codon immediately upstream of the protease sequence, indicate that the F6A *pol* proteins are translated as a 1,709-amino-acid (180-kDa) precursor protein containing *gag* proteins at the N terminus. MuLV produce an analogous 180-kDa *gag-pol* polyprotein by a similar in-frame, stop codon suppression mechanism (8, 67).

Protease, reverse transcriptase, and endonuclease-integrase boundaries within F6A *pol* may be inferred by alignment with MuLV *pol* genes and comparison with the known N- and C-terminal residues of MuLV reverse transcriptase (6, 22, 57). The deduced primary sequence of MuLV protease shares 82% homology with F6A, and regions spanning proteolytic cleavage sites in *pol* and *gag* are conserved in F6A and MuLV, suggesting that processing of F6A proteins may occur at these conserved sites. Cleavage at the predicted recognition sites of F6A *pol* products would generate a 125-amino-acid (13-kDa) protease, a 667-amino-acid (74-kDa) reverse transcriptase (the molecular mass is similar to the reported 72-kDa molecular mass of an FeLV reverse transcriptase [50]), and a 416-amino-acid (47-kDa) endonuclease-integrase. In agreement with these predictions, antibodies against MuLV *pol* products identify cross-reactive FeLV proteins with molecular weights similar to those of the

FIG. 3. Alignment of deduced FeLV *gag* gene products and the protease protein of *pol*. The F6A sequence is used as a reference. Abbreviations: FeSV-GA, FeSV-Gardner-Arnstein (19); FeSV-SM, FeSV-Susan McDonough (18); FeSV-ST, FeSV-Snyder Theilen (19); p10/prot, p10 primary sequence (7) and a portion of the protease primary sequence (66). Deduced N-terminal positions of each *gag* precursor protein (gPr80 and Pr65), mature *gag* proteins (p15, p12, p30, p10), and *pol* protease are indicated. Sequences not determined are indicated by dots.

predicted F6A reverse transcriptase and endonuclease-integrase proteins (60).

Alignment of F6A with partial *pol* sequence data from other FeLV clones indicates strong amino acid conservation. All 125 amino acids of the deduced FSB protease protein are homologous (if the aforementioned cytosine is deleted from FSB) (Fig. 3) (35), and 264 of 265 deduced amino acids at the 3' end of FGB *pol* are conserved (10; J. Elder, personal communication).

Conservation of FeLV envelope gene sequences. Retroviral envelope proteins are translated from a spliced subgenomic mRNA containing sequences from the 5' end of the viral genome and coding sequences derived from the envelope

ORF in the 3' third of the genome (12, 36). The F6A *env* mRNA could be generated by using the consensus splice donor at position 542 and the splice acceptor at position 5669. Both are homologous to the identified splice sites of Moloney MuLV (36). The predicted start of translation in *env* (position 5981) is not the most proximal methionine to the splice acceptor site; an additional AUG codon occurs at position 5911, but is followed immediately by a stop codon. The amino termini of FeLV gp70 and p15E polypeptides have previously been inferred by alignment with known MuLV termini (10, 46, 51, 59, 65). These regions bear strong homology to sequences within F6A and F3A, suggesting that both *env* genes encode a 33-amino-acid leader sequence

beginning at position 5981 in F6A, a 412-amino-acid gp70 beginning at position 6080, and a 197-amino-acid p15E protein beginning at position 7316.

All of the known, deduced FeLV p15E proteins are highly conserved, differing by 1 to 3% at the amino acid level (data not shown). However, deduced gp70 amino acid sequences from FeLV of all three subgroups show more extensive divergence. Figure 4 displays an alignment of the F6A gp70 with that of F3A, FGA, FSC, and FGB. FSC is 88% homologous to F6A and has a 3-amino-acid deletion. FGB is 80% homologous; two large insertions, a 1-amino-acid insertion, and two deletions must be introduced to maintain alignment. In striking contrast, only 9 residues of FGA and 11 residues of F3A differ from the deduced F6A gp70 sequence, with no insertions or deletions; this corresponds to 98% homology. Of these substitutions within FGA and F3A, seven (77%) and four (36%), respectively, are conservative. The positions of all 16 cysteine residues are conserved among the three deduced sequences; and all 10 potential N-linked glycosylation sites are conserved.

Relatedness of F6A to other retroviruses. Comparison of deduced F6A amino acid sequences with those of MuLV demonstrate, as expected (4, 5), that FeLV and MuLV are very closely related. The predicted endonuclease domains of F6A and Moloney MuLV or AKV MuLV (22, 57) are 67 to 68% homologous, with two insertions (6 and 2 amino acids). Within reverse transcriptase, these viruses are 76% homologous, with four sites of single-amino-acid insertions (Fig. 5). In agreement with previous studies which based genetic

relatedness on the degree of nucleic acid hybridization (4, 5), other known retroviruses share less deduced protein sequence homology. For example, simian sarcoma virus is 59% homologous to the deduced F6A endonuclease and avian reticuloendotheliosis virus is 40% homologous. Rous sarcoma virus, human T-cell lymphotropic virus type I, human immunodeficiency virus, equine infectious anemia virus, Mason-Pfizer type D virus, and mouse mammary tumor virus type B share between 20 and 30% homology (data not shown) with F6A.

Alternative ORFs. F6A and F3A contain a number of short ORFs (Fig. 2). To determine whether any might encode functional proteins (although none have as yet been identified), potential splice donors, splice acceptors, and methionines were located. A total of 10 potential splice donors and more than 20 potential acceptors were identified. Four ORFs could encode methionine-initiated polypeptides as large as 51, 48, 42, and 35 residues, but none contained consensus 3' splice donor sites to allow fusion with additional exons. One ORF contained a 34-amino-acid coding segment with a 5' methionine and a 3' splice donor that might splice in frame with potential splice acceptor sites in three other ORFs to generate ORFs of 80, 74, or 99 amino acids. Nevertheless, low-stringency alignment of a three-frame translation of clone F6A and the *trans*-activator polypeptides from human immunodeficiency virus type 1 (2, 58), the reported alternate reading frame product in the Rous sarcoma virus *gag* gene (5), and potential Moloney MuLV short ORF products did not reveal any regions of substantial homology (data not

FIG. 4. Alignment of deduced FeLV gp70 proteins. The F6A sequence is used as a reference. Divergent amino acids are indicated above and below the F6A sequence. Potential sites of glycosylation are underlined.

FIG. 5. Alignment of deduced F6A and Moloney MuLV reverse transcriptase proteins (57). Divergent MuLV residues are indicated below the reference F6A sequence.

shown). The same results were obtained with the partial F3A sequence, which has a nearly identical ORF arrangement to F6A.

Biological activity of F3A. F3A clone 50, which was used for DNA sequence analysis, was unable to encode infectious virus following transfection of feline embryo fibroblasts (Table 1). This defect mapped 5' of the *env* gene, since a chimeric virus generated in vitro, containing the *env*-3'-LTR region of this clone, yielded infectious virus, whereas the reciprocal construct containing the *gag-pol* region did not (Table 1). In contrast, F3A clone 2 produced infectious virus in this assay and was used for inoculation into specific-pathogen-free cats. F3A clone 2 induced viremia in each of three weanling-age (8-week-old) and four newborn cats, none of which had developed symptoms of disease 6 months postinoculation (Table 1). In addition, 12 cats inoculated with F6A became viremic and did not develop disease within 1 year of inoculation (Table 1). All animals inoculated with F3A clone 2 or F6A became viremic between 2 and 3 weeks postinoculation. Thus, the F3A virus, like F6A, corresponds to a minimally pathogenic FeLV.

DISCUSSION

The deduced gp70 proteins of F6A, F3A, and FGA share remarkably strong (98%) homology, despite their isolation from naturally infected cats up to 13 years apart and from widely separate geographic locations: FGA was isolated in Glasgow, Scotland, in 1970 (30) and carried in culture for several years before being subjected to molecular cloning (59); F3A was isolated in New York City in 1977 (E. Zuckerman and W. D. Hardy, Jr., personal communication) and was also extensively propagated in culture before being cloned; and F6A was molecularly cloned from cat tissue DNA after one in vivo passage of a virus isolated from a pet cat in Fort Collins, Colo. in 1983 and was never propagated in vitro before being cloned (26; Overbaugh et al., submit-

ted). All three proviruses were molecularly cloned and sequenced in different laboratories. This remarkable sequence conservation may reflect stringent selection against antigenic changes in the ubiquitous, viremia-inducing and horizontally transmissible form of FeLV. Neutralization studies have previously demonstrated antigenic stability among subgroup A viruses in cats from diverse geographic locations (53). This is in greatest contrast to the high degree of divergence observed among animal lentiviruses (16) and human immunodeficiency virus isolates (39, 64). Although continual generation of antigenic variation may provide a mechanism for escape from immunity, F6A-like viruses may, conversely, use a mechanism by which a minimally immunogenic envelope structure is maintained to escape immunological recognition.

The stability of envelope sequences in F6A, F3A, and FGA is also remarkable when compared with the closely related MuLV. For example, two ecotropic MuLV isolates, Moloney and AKV, share only 80% homology within gp70, versus 98% shown here for three minimally pathogenic FeLVs (22, 57).

It is generally accepted that envelope glycoproteins determine virus subgroup (62). The remarkable similarity of the F6A, F3A, and FGA gp70s, together with their relative dissimilarity from subgroup B and C gp70s, strongly argues that F6A and F3A belong to FeLV subgroup A. As described here and elsewhere (Overbaugh et al., submitted), F6A and F3A induce viremia, but not acute immunodeficiency or other disease when inoculated into specific-pathogen-free cats. These features are characteristic of subgroup A, but not subgroup B or C, FeLVs (28, 32, 51). Therefore, F6A represents a prototype of the highly conserved, horizontally transmitted, minimally pathogenic subgroup A form of FeLV that is probably present in all naturally infected cats.

The F6A nucleotide sequence provides a basis for comparison with individual pathogenic FeLV isolates to identify potentially important disease determinants. The analyses

presented here indicate that major structural changes, occurring primarily in gp70, distinguish the pathogenic subgroup B and C viruses from the monotypic subgroup A viruses. The importance of these differences may be evaluated by constructing chimeric viruses between pathogenic viruses and the most completely characterized and minimally pathogenic F6A clone. We are currently using this approach to identify the determinants for induction of feline acquired immune deficiency syndrome and aplastic anemia in cats.

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