Pathogenic and host range determinants of the feline aplastic anemia retrovirus

(feline leukemia virus subgroup C/extracellular glycoprotein)

NORBERT RIEDEL*†, EDWARD A. HOOVER‡, RONNA E. DORNSIFE‡, AND JAMES I. MULLINS*§

*Department of Cancer Biology, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115; and ‡Department of Pathology, Colorado State University, Fort Collins, CO 80523

ABSTRACT   Feline leukemia virus (FeLV) C-Sarma (or FSC) is a prototype of subgroup C FeLVs, which induce fatal aplastic anemia in outbred specific-pathogen-free (SPF) cats. FeLV C isolates also possess an extended host range in vitro, including an ability, unique among FeLVs, to replicate in guinea pig cells. To identify the viral determinants responsible for the pathogenicity and host range of FSC we constructed a series of proviral DNAs by exchanging gene fragments between FSC and FeLV-61E (or F6A), the latter of which is minimally pathogenic and whose host range in vitro is restricted to feline cells. Transfer of an 886-base-pair (bp) fragment of FSC, encompassing the codons for 73 amino acids at the 3' end of pol (the integrase/endonuclease gene) and the codons for 241 amino acids of the N-terminal portion of env [the extracellular glycoprotein (gp70) gene], into the F6A genome was sufficient to confer onto chimeric viruses the ability to induce fatal aplastic anemia in SPF cats. In contrast, no chimera lacking this sequence induced disease. When assayed in vitro, all chimeric viruses containing the 886-bp fragment of FSC acquired the ability to replicate in heterologous cells, including dog and guinea pig cells. Thus, the pathogenic and the host range determinants of the feline aplastic anemia retrovirus colocalize to a 3' pol–5' env region of the FSC genome and likely reside within a region encoding 241 amino acid residues of the N terminus of the extracellular glycoprotein.

Aplastic anemia (AA) in cats is a naturally occurring disease characterized by severe normochromic nonregenerative anemia, lymphopenia, and, in its later stages, granulocytopenia and medullary osteosclerosis of the long bones (1, 2). We have recently shown that molecular clone-derived feline leukemia virus (FeLV) C-Sarma, a prototype of the C subgroup of feline leukemia retrovirus and henceforth referred to as FSC, reproducibly induces viremia, depletion of erythroid stem cells, and fatal AA within 8 weeks of inoculation of genetically outbred, specific-pathogen-free (SPF) cats (3). Thus, the determinants for the induction of irreversible erythroid aplasia are encoded within the FSC genome. Furthermore, subgroup C isolates of FeLV are the only retroviruses known to induce AA (2, 4); therefore, FSC provides a unique model for study of the pathogenic mechanisms of this disease.

The pathogenic spectrum of feline AA is similar to that of severe AA in humans. Prognosis for patients with AA is poor: 90% of individuals diagnosed with AA die within 15 years (5). Certain drugs and chemicals are known etiological agents of acquired AA (6); however, infection with hepatitis A virus or Epstein–Barr virus has also been associated with human AA (7, 8). A possible viral origin of the disease is further supported by studies demonstrating that human parvovirus causes transient marrow aplasias in children (9).

Nevertheless, FeLV C is the only virus known to induce fatal, nonregenerative anemia in any species.

Some pathogenic determinants of retroviral genomes have been localized to the env–long terminal repeat (LTR) region. Studies with subgroup F avian leukosis viruses suggest that env gene sequences confer the ability to induce angiosarcoma in chickens (10). In the replication-defective murine Friend spleen focus-forming virus (SFFV), the env gene appears to confer specificity for virus-induced proliferation of erythroid precursor cells (11–13). Nevertheless, env genes appear not to be universally responsible for disease specificity. It has been suggested that sequences within the ecotropic murine leukemia virus (MuLV) LTR determine cell tropism and leukemogenic potential, possibly due to tissue-specific enhancer activity (14–17). However, more recent analyses of Moloney and Friend MuLVs indicate that the effect of these LTRs on tissue-specific replication is restricted to a very early preleukemic stage of disease, whereas at later times after infection structural gene sequences predominantly influence tissue-specific replication (18). Moreover, studies by Sitbon et al. (19) demonstrate that, in Friend MuLV, determinants for hemolytic anemia and erythroleukemia map to a pol–env fragment and the LTR-gag region, respectively. In avian leukosis virus, the potential to induce osteopetrosis is determined by sequences in the gag region near the 5' LTR, possibly involving regulation at the level of viral gene expression and virus maturation (20, 21). Involvement of multiple genes in disease induction has also been suggested by studies with the murine retrovirus MCF 247, where maximum oncogenic potential and shortened latency of disease induction in AKR mice depend on at least four viral genes, each of which alone either is ineffective or only moderately enhances oncogenicity (22).

Another property that distinguishes retroviruses is host range of infection in vitro, thought to be determined in most cases by cell receptor recognition of the major extracellular glycoprotein (23). The FeLV family consists of three known subgroups, A, B, and C, defined by viral interference and possibly differing in host range (24, 25). While most FeLV A isolates are restricted to growth in feline cells, FeLV C isolates replicate in a variety of heterologous cells and are unique, as far as we know, in their ability to replicate in guinea pig fibroblasts (24, 25).

To identify regions within the FSC genome crucial for the induction of AA and to localize the host range determinants of this virus, we constructed chimeric proviruses between anemogenic FSC (3) and the minimally pathogenic F6A

(subgroup A) (26) by exchanging complete genes or gene fragments. Here we report that a chimeric F6A, containing an 886 base-pair (bp) fragment of FSC encoding 72 amino acids of the C terminus of the presumed integrase/endonuclease protein (the 3' end of the *pol* open reading frame) and 241 amino acids of the N-terminal region of the extracellular glycoprotein, confers the specificity for induction of AA indistinguishable from that induced by parental FSC. None of the chimeras that lack this sequence induce AA. Furthermore, the chimeras that induce AA also acquire the unique host range properties of FSC. Thus, the N-terminus-encoding portion of the FSC gp70 gene likely encodes the major determinant of both its lineage-specific pathogenicity *in vivo* and its characteristic host range *in vitro*.

MATERIALS AND METHODS

Construction of Chimeric Viruses. The molecular cloning, biological properties, and nucleotide sequence of the *env*-LTR region of FSC and of the complete genome of F6A are described elsewhere (3, 26–28).

Restriction sites used to construct chimeras between these two viruses were as follows: *Xho* I, located in the *pol* open reading frame 162 bp 5' to the beginning of the gp70 gene; *Sst* II, located within the p15E gene 59 bp from the codon for its N terminus; *Bcl* I, positioned within the gp70 gene; and *Eco*RI and *Xba* I, both located within the plasmid vector.

To exchange gp70 genes, plasmid clones pFSC-env-LTR (3) and the analogous pF6A-env-LTR (26) were digested with *Sst* II (in the p15E gene) and *Xba* I (in vector) and the respective insert and vector fragments were ligated. Exchange of 886-bp *Xho* I/*Bcl* I fragments encoding 73 amino acids of integrase/endonuclease and the N-terminal 241 amino acids of gp70 as well as 665-bp *Bcl* I/*Sst* II fragments encoding the C-terminal 201 amino acids of gp70 and the N-terminal 21 amino acids of p15E was carried out in a similar fashion. Plasmids pFSC-env-LTR and pF6A-env-LTR were first amplified in the *dam*⁻ *Escherichia coli* strain GM48 to allow subsequent cleavage by *Bcl* I. The genome structure of all reconstructed viruses was verified by using restriction enzyme sites uniquely positioned in the exchanged fragments.

Transfection Experiments and *in Vitro* Host Range Studies. Cell lines examined include feline embryo fibroblasts AH927, D-17c-1 canine osteosarcoma (3), and GP104 guinea pig fibroblasts (ATCC 158). Cells were transfected by electroporation (29) at 1000–2000 V, using 1.0×10^6 cells per ml and 5 μg of plasmid DNA or were infected by incubating cell monolayers for 1.5 hr with cell-free supernatants (filtered through 0.22-μm-pore nitrocellulose filters) from virus-producing cells. In all cases, parental viruses served as controls for transfection or infection experiments. Cells were monitored for production of virus by reverse transcriptase assays (30) and enzyme-linked immunosorbent assay (ELISA) for p27-gag (Virachek, Synbiotics, San Diego, CA) at weekly intervals after transfection or infection for up to 4 weeks.

Animal Inoculation Studies. All *in vivo* studies were carried out with littermate uninoculated cats as controls. Separate litters were used to evaluate each individual construct. Subgroup C viruses induce viremia or disease in newborn cats but are incapable of inducing viremia or disease in weanling cats when inoculated intraperitoneally (3). We therefore employed two different routes of infection to establish viremia in weanling cats: (*i*) inoculation of isolated homologous bone marrow mononuclear cells after *in vitro* infection with cell-free supernatants from virus-producing feline (for F6A and construct S-S-E-E) or canine cells and (*ii*) direct inoculation of short-term virus-infected feline embryo fibroblasts into the bone marrow of recipient cats (unpublished data). These inocula-

tion procedures were developed to bypass the age-related resistance of weanling cats and thereby circumvent the necessity of using rarely available, hematologically immature, and expensive newborn animals and permit consistent induction of persistent viremia so that pathogenic activity of each virus could be assessed efficiently.

To determine the capacity of recombinant retroviruses to induce AA, a total of 71 weanling age (8 to 13 weeks) SPF cats from a breeding colony at Colorado State University were inoculated with 5×10^7 virus-producing feline embryo fibroblasts and 42 animals became viremic. At biweekly intervals after inoculation, blood and bone marrow cells were collected from each cat and methanol-fixed films were prepared for the detection of FeLV viral structural antigen p27 in leukocytes, platelets, and marrow cells by immunofluorescence (3). Hemograms were obtained at most sampling intervals to detect the presence of anemia or other hematologic abnormalities. Clonogenic methylcellulose colony-forming assays for erythroid and granulocyte–macrophage progenitor cells in bone marrow were performed at various intervals after inoculation by procedures described previously (3). All cats were euthanized by barbituate overdose when signs of severe nonregenerative anemia were evident.

DNA Analysis. Isolation of DNA and Southern transfer and hybridization analyses were performed as described previously (3, 31). DNAs were digested with restriction enzyme combinations that allowed unambiguous identification of recombinant viruses after hybridization with an exogenous LTR-specific probe (J.I.M., unpublished data; ref. 31) or a full-length FeLV probe (3).

RESULTS

Biological Properties of Parental and *env*-LTR Recombinant Viruses. The first series of experiments compared the pathogenicity of parental viruses FSC (S) and F6A (E) with two recombinants (Fig. 1; E-E-S-S, S-S-E-E) constructed by cleaving the viral genome at the unique *Xho* I site near the 3' end of the *pol* region, 162 bp upstream of the gp70 start codon. Due to the process of retroviral replication (32), the R-U5 regions of the daughter virus are derived from the parental virus 5' LTR, while the U3 region, providing promoter and enhancer function for the progeny virus, is derived from the parental virus 3' LTR.

Canine cells, which lack FeLV-related endogenous sequences, were used to propagate anemogenic FeLVs to exclude the possibility that pathogenic viruses were generated by endogenous recombination *in vitro*. Furthermore, in all of the studies reported here, Southern blot analysis of viral DNA derived from cell lines and from terminal cat tissues revealed no viral gene rearrangement (data not shown).

FSC induced viremia in 13 weanling-age cats, all of which died of AA within 8 weeks of inoculation. As previously described (3), AA was defined by a decrease in hematocrit in the absence of hematologic indicators of regeneration—namely, macrocytosis, polychromasia, and reticulocytosis—and by a precipitous decline in marrow erythroid progenitor colony-forming cells (burst-forming units, BFU-e) in marrow clonogenic assays (relative to an uninoculated littermate control).

By contrast, 16 animals inoculated with F6A rapidly became viremic but have not developed disease more than 14 months after inoculation. Chimeric virus E-E-S-S, containing the 5' R-U5-*gag-pol* region of F6A and the entire *env*-U3 region of FSC (Fig. 1), induced viremia in 3 animals, and all died of AA within 8 weeks of inoculation. The reciprocal construct (S-S-E-E) induced viremia in 2 inoculated animals, both of which have remained asymptomatic for more than 14 months. These results indicate that the

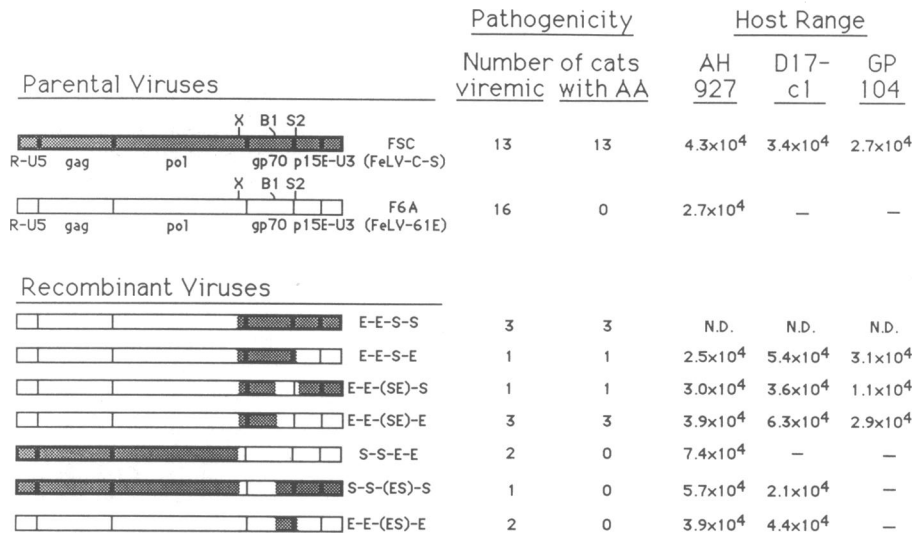| Parental Viruses	Pathogenicity		Host Range		
	Number of cats viremic	with AA	AH 927	D17-c1	GP 104
FSC (FeLV-C-S)	13	13	4.3×10^4	3.4×10^4	2.7×10^4
F6A (FeLV-61E)	16	0	2.7×10^4	—	—
Recombinant Viruses					
E-E-S-S	3	3	N.D.	N.D.	N.D.
E-E-S-E	1	1	2.5×10^4	5.4×10^4	3.1×10^4
E-E-(SE)-S	1	1	3.0×10^4	3.6×10^4	1.1×10^4
E-E-(SE)-E	3	3	3.9×10^4	6.3×10^4	2.9×10^4
S-S-E-E	2	0	7.4×10^4	—	—
S-S-(ES)-S	1	0	5.7×10^4	2.1×10^4	—
E-E-(ES)-E	2	0	3.9×10^4	4.4×10^4	—

FIG. 1. Pathogenicity and host range of parental and chimeric viruses. Viruses are described by using the capital letters S for FSC and E for F6A to designate segments contributing the following regions of the viral genome: R-U5-*gag, pol*, gp70, and p15E-U3. Only those restriction sites (of the corresponding proviruses) used in the generation of chimeric viruses are shown. Enzyme abbreviations are X, *Xho* I; B1, *Bcl* I; and S2, *Sac* II. The precise positions of restriction enzyme recognition sites relative to gene boundaries are described in *Materials and Methods*. For chimeric viruses containing intragenic gp70 switches, regions of FSC or F6A that were placed into the respective gp70 gene appear in parentheses and are named according to the origin of the intragenic fragment. Inoculation of weanling cats and serial examinations for viremia and onset of disease are described in the text. Host range of parental and *in vitro* reconstructed FeLVs was determined by using AH927 feline embryo fibroblasts, D-17c-1 canine cells, and GP104 guinea pig fibroblasts. These cells were transfected, infected, or both and monitored for production of virus by reverse transcriptase assay and ELISA 30 days after transfection or infection. Reverse transcriptase activity is expressed as cpm per assay above background (<500 cpm), corresponding to 2 ml of cell supernatant. N.D., not done. A — indicates background level. Results for ELISA paralleled those for reverse transcriptase (not shown).

anemogenic potential of FSC is determined by sequences 3' to the *Xho* I site, likely within the *env*-U3 region of the genome.

Pathogenic Determinants Within *env*-LTR. To identify the regions within the *env*-LTR portion of the FSC genome essential to induction of AA, we transferred a *Xho* I/*Sst* II fragment, containing the 3'-terminal *pol* region and the entire gp70-encoding region of FSC, into F6A. This fragment also encodes the first 19 amino acids of the *env*-encoded p15E transmembrane protein; these residues are identical in the two viruses and therefore could not confer the unique pathogenicity of FSC. As summarized in Fig. 1, construct E-E-S-E induced AA. No differences in latency of disease induction or progression were observed when comparing FSC, E-E-S-S, and E-E-S-E, indicating that the region

encompassing the 3' *pol*–gp70 gene region of FSC alone conferred the specificity for the induction of AA.

A unique *Bcl* I site within the gp70 gene was used to construct intragenic recombinants between FSC and F6A in an attempt to further delimit the viral determinants for induction of AA. An 886-bp *Xho* I/*Bcl* I fragment, encoding the C-terminal 73 amino acids from the viral integrase/endonuclease and the N-terminal 241 amino acids of gp70, and a 665-bp *Bcl* I/*Sst* II fragment, encoding the C-terminal 201 amino acids of gp70, were exchanged (Fig. 2). After reconstruction of complete proviruses and transfection of D-17c-1 canine cells, chimeric viruses E-E-(SE)-E, E-E-(ES)-E, E-E-(SE)-S, and SS-(ES)-S were inoculated into weanling cats, which were then monitored for induction of viremia and onset

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                -->3'-pol
                XhoI                                           3'-pol<--|
FELV-C-Sarma    LEPRWKGPHIVLLTTPTALKVDGVAAWIHASHVKAAGPTTDQDLPNDPSSDDPSRWKVQRTQNPLKIRLSRGT
FELV-61E                                                       N      SDS


                                            |-->gp70-leader             |-->gp70
FeLV-C-Sarma    MESPTHPKPSKDKTFPWNLVFLVGILFQIDMGMANPSPHQVYNVTWVITNVQTNSRANATSMLGTLTDAYPTLYVD
FeLV-61E                  LS          T I         I             TQ             V    H


FELV-C-Sarma    LCDLVGDTWEPIAPDPRS----WARYSSSTHGCKTTDRKKQQQTYPFYVCPGHAPSMGPKGTYCGGAQDGFCAAWGCETTGEAWWKPTSSWDYITVKRGSNQDNSCKGKCNPLVLQFTQKGRQASWDRPKM
FELV-61E                   VLS TNVKHG  P KY               L    H                       S            S  N E    I    K    G
                    VR1

                              BclI
FELV-C-Sarma    WGLRLYRSGYDPIALFSVSRQVMTITPPQAMGPNLVLPDQKPPSRQSQTKSKVTTQRPQITSSTPRSVASATMGPKRIGTGDRLINLVQGTYLALNATDPNKTKDCWLCLVSRPPYYEGIAVLGNYSNQT
FELV-61E            T        T     S                 G  A   TNE A   PT V                                                                   I
                                                    VR4

                                                          |-->p15E         SstII
FELV-C-Sarma    NPPPSCLSTPQHKLTISEVSGQGLCIGTVPKTHQALCKKTQKGHKGTHYLAAPNGTYWACNTGLTPCISMAVLNWTSDFCVLIELWPRVTYHQPEYIYTHFDKAVRFRREPISLTVALMLGGLTVGGIAA
FELV-61E                       I                       N  Q T A                                          V    A
                                                    VR5
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FIG. 2. Deduced amino acid sequence of the 3' *pol* and *env* region of FSC and F6A. The standard one-letter code is used. The region of *pol* 3' to the *Xho* I site, the gp70 gene, and the 5' region of p15E up to the *Sst* II site are indicated. The *Bcl* I site within the gp70 gene was used to subdivide this gene. Amino acids of F6A not shared with FSC are indicated below the FSC sequence; a gap, indicated by a broken line, is introduced to maintain alignment. Numbering of the variable regions (stippled areas) is based on comparison of nine FeLV isolates of different pathogenic potential (J.I.M., unpublished results); only three of the five variable regions identified differ between FSC and F6A (vr1, vr4, and vr5).

of AA. Each cat that developed persistent viremia after inoculation with E-E-(ES)-E ($n = 2$) and S-S-(ES)-S ($n = 1$) remained asymptomatic for an observation period of 8 months after inoculation (Fig. 1), strongly suggesting that regions outside of the *Xho* I/*Bcl* I fragment do not contribute to the unique pathogenicity of FSC. In contrast, all of four cats in which constructs E-E-(SE)-E and E-E-(SE)-S induced viremia developed AA with hematologic characteristics and latency indistinguishable from those associated with FSC, thus indicating that the region of the genome encoding the N-terminal portion of gp70 is the major determinant for the disease specificity of FSC.

Comparison of the *env*-LTR sequences of nine FeLV proviruses of different pathogenic potential reveals that the p15E genes and LTRs are highly homologous (J.I.M., unpublished results). However, we have identified up to five blocks of clustered amino acid changes and deletions/insertions in the deduced gp70 proteins, which we have defined as variable regions (vr) 1 to 5 (J.I.M., unpublished results). Three of these variable regions, designated vr1, vr4, and vr5 (stippled areas in Fig. 2), constitute the most substantial differences between the extracellular glycoproteins of FSC and F6A. The first variable region, vr1, is found within the fragment specifying the determinant for AA. Secondary structure predictions based on the Chou–Fasman algorithm (33) suggest that an α-helical structure is uniquely favored in this region of FSC relative to F6A (not shown). The other variable regions that distinguish FSC and F6A are found in the sequence encoding the C-terminal region of gp70, outside of the region encoding the pathogenic determinant (vr4 and vr5, stippled in Fig. 2).

In Vitro **Host Range of Intra-gp70 Recombinants.** FeLV subgroups differ in their host range of infection *in vitro* (24, 25). Only FeLV C replicates well in feline, canine, and guinea pig cells, whereas FeLV A, including F6A, replicates well in feline cells and very poorly or not at all in canine and guinea pig cells, respectively (Fig. 1). Cell receptor recognition of the virion envelope glycoprotein, a product of the *env* gene, is thought to be one important component determining host range of retroviruses (23).

The parental and chimeric viruses described above were transfected into feline, canine, and guinea pig cells and tested for extracellular reverse transcriptase activity and viral antigen p27-gag for up to 30 days after transfection. F6A as well as S-S-E-E (Fig. 1) and S-S-E-S (not shown) replicated well in feline cells but did not replicate in canine cells or guinea pig cells, in contrast to FSC and E-E-S-E, which replicated in each of the three host cell lines. These results indicate that the unique extended host range determinants of FSC reside within the extracellular glycoprotein. Of the intragenic recombinant viruses, only E-E-(SE)-S and E-E-(SE)-E replicated in canine and guinea pig cells, while constructs S-S-(ES)-S and E-E-(ES)-E had an intermediate host range and replicated well in canine but not in guinea pig cells. Guinea pig cells also remained resistant to infection when incubated with cell-free supernatants from S-S-(ES)-S- and E-E-(ES)-E-infected canine cells. These data suggest that cell surface receptor recognition by FeLV can involve interaction of domains within gp70 and that combinations of multiple domains can result in altered host range properties. Moreover, these results indicate that a region including the N-terminal portion of FSC gp70, already implicated as a major pathogenic determinant, is also an essential component determining the host range of FeLV C *in vitro*.

DISCUSSION

This study demonstrates that an 886-bp region of the FSC genome, encoding 73 amino acids of the C terminus of the *pol* gene product and 241 amino acids of the N terminus of

gp70, is the major determinant for induction of AA in outbred SPF cats. The R-U5-*gag-pol* and the p15E-U3 regions of FSC can be replaced by the corresponding regions of F6A without altering the anemogenic potential, suggesting that these regions do not confer pathogenic potential or that crucial functions in addition to the FSC gp70 determinant can be provided by the corresponding sequences of nonanemogenic F6A. Although in some cases constructs were tested in only one or two viremic cats, we have never observed inconsistency in the pathogenic outcome of infection with any of the viruses under study (see Fig. 1).

Possible mechanisms we have considered for the abnormal control of hematopoiesis leading to induction of fatal AA include a cytopathic or cytostatic effect of FSC and anemogenic chimeras on erythroid-committed stem cells and virus-induced defects in accessory cells in the supporting bone marrow microenvironment. Among the earliest events observed after virus inoculation is the suppression of proliferation or differentiation of early bone marrow erythroid progenitor cells (BFU-e) (3). Although expression of stem cell surface receptors may vary during commitment and differentiation, a receptor(s) utilized by FSC and anemogenic chimeric viruses does not seem to be exclusively expressed on erythroid-committed cells, because these viruses also infect other cells of the cat hemopoietic system (e.g., megakaryocytes, neutrophil progenitors) and various tissue cells, including spleen, lymph node, salivary gland, and intestine, as well as fibroblasts and T cells *in vitro* (ref. 3; data not shown). Therefore, virus-induced suppression of proliferation or differentiation of erythroid-committed stem cells is not necessarily the result of gp70-mediated target cell selection, and receptor binding or intracellular expression of the FSC gp70 may have a unique effect upon these or accessory cells—e.g., by inhibiting the binding of essential hematopoietic growth factors supplied by the supporting microenvironment or by specifically interfering with regulatory processes within erythroid-committed cells.

That FeLV infection and replication in erythroid-committed cells does not lead to cytopathic effect *per se* is suggested by our finding that BFU-e of cats infected with F6A are antigen-positive (E.A.H., unpublished results), yet these animals remain asymptomatic. However, given their unique receptor recognition properties, FSC and F6A could interact with different receptors on the same erythroid stem cell.

The first variable region contained within the gene for the N-terminal portion of the FSC gp70 (Fig. 2) may encode the crucial determinant for disease induction. However, there are 25 additional amino acid changes encoded within the 886-bp *Xho* I/*Bcl* I fragment, of which 17 fall into the mature gp70 protein, 4 within the envelope leader peptide, and 4 within the product of the 3' end of the *pol* open reading frame (Fig. 2). The portion of *pol* encoding 73 amino acid residues of the C terminus, by analogy with murine retroviruses, likely codes for a portion of the presumed integrase/endonuclease protein (34, 35), and the 4 nonconservative amino acid changes in this region between FSC and F6A (Fig. 2) could conceivably affect proviral integration, resulting in persistent unintegrated viral DNA (UVD), which is associated with cytopathic retrovirus infections (36). However, no significant levels of UVD were detected in virus-infected cell lines or bone marrow of 24 cats at various intervals after inoculation with FSC and anemogenic chimeric viruses (data not shown). Furthermore, accumulation of UVD has not been associated with *pol* gene mutants in other systems (37). It is therefore unlikely that the observed changes in the FSC *pol* gene play a role in the production of cytopathic levels of UVD.

Host range properties of retroviruses are thought to be determined primarily by cell receptor recognition of the *env* gene product (23), and recent studies with avian retroviruses

suggest that several variable regions within gp85 are involved in determining cell receptor interaction (38). Our results also indicate that FeLV gp70 domains interact in determining receptor binding properties. Construct E-E-(SE)-E, like FSC, replicated in canine and guinea pig cells, demonstrating that the unique host range of subgroup C FeLVs can be mapped to the N-terminal portion of gp70 (Fig. 1). However, chimeric viruses E-E-(ES)-E and S-S-(ES)-S, containing the N-terminal portion of gp70 from F6A and the remainder of gp70 from FSC, displayed an intermediate host range and replicated in canine cells but not in guinea pig cells, suggesting that distant gp70 domains can interact in cell receptor recognition. The predicted polypeptide sequences of variable regions 1, 4, and 5 (Fig. 2) appear hydrophilic and are therefore likely to be localized on the surface of gp70, and they may be involved in receptor recognition. Furthermore, colocalization of the pathogenic and unique host range determinants of FSC suggests that the unique receptor recognition properties play a role in the pathogenicity of this virus.

Comparison of the pathogenic spectrum of feline and human AA and recent identification of hepatitis A virus (7) and Epstein–Barr virus (8) in acquired AA and of human parvovirus in transient marrow aplasias in children (9) indicate that hemolymphotropic viruses may be implicated in marrow aplasias in humans (7–9). Since subgroup C FeLVs are the only retroviruses known to induce nonregenerative AA, they represent a unique model system to study mechanisms and pathways of stem cell suppression.

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