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Identification of Three Feline Immunodeficiency Virus (FIV) *env* Gene Subtypes and Comparison of the FIV and Human Immunodeficiency Virus Type 1 Evolutionary Patterns

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Feline immunodeficiency virus (FIV) is a lentivirus associated with AIDS-like illnesses in cats. As such, FIV appears to be a feline analog of human immunodeficiency virus (HIV). A hallmark of HIV infection is the large degree of viral genetic diversity that can develop within an infected individual and the even greater and continually increasing level of diversity among virus isolates from different individuals. Our goal in this study was to determine patterns of FIV genetic diversity by focusing on a 684-nucleotide region encompassing variable regions V3, V4, and V5 of the FIV *env* gene in order to establish parallels and distinctions between FIV and HIV type 1 (HIV-1). Our data demonstrate that, like HIV-1, FIV can be separated into distinct envelope sequence subtypes (three are described here). Similar to that found for HIV-1, the pairwise sequence divergence within an FIV subtype ranged from 2.5 to 15.0%, whereas that between subtypes ranged from 17.8 to 26.2%. However, the high number of synonymous nucleotide changes among FIV V3 to V5 *env* sequences may also include a significant number of back mutations and suggests that the evolutionary distances among FIV subtypes are underestimated. Although only a few subtype B viruses were available for examination, the pattern of diversity between the FIV A and B subtypes was found to be significantly distinct; subtype B sequences had proportionally fewer mutations that changed amino acids, compared with silent changes, suggesting a more advanced state of adaptation to the host. No similar distinction was evident for HIV-1 subtypes. The diversity of FIV genomes within individual infected cats was found to be as high as 3.7% yet twofold lower than that within HIV-1-infected people over a comparable region of the *env* gene. Despite these differences, significant parallels between patterns of FIV evolution and HIV-1 evolution exist, indicating that a wide array of potentially divergent virus challenges need to be considered in FIV vaccine and pathogenesis studies.

The study of lentiviral diversity has generally focused on the envelope (*env*) gene, the most variable structural gene (32). The Env protein of human immunodeficiency virus type 1 (HIV-1) is an important antigenic target of the virus and encodes the principal neutralizing determinant (12, 26). HIV-1 sequences can be separated into phylogenetically distinct envelope sequence subtypes (32). To date, seven HIV-1 subtypes have been identified: subtypes A and D are found predominately in central Africa; subtype B is common in the United States and western Europe; subtype C is common in southern Africa, East Africa, and India; subtype E is common in Thailand and has been found in the Central African Republic; subtype F has been found in Brazil and Romania; and the newest subtype, O, was found in Cameroon (7, 24, 29, 32). Because of the potential need to generate geographically and temporally specific vaccine formulations, investigations to determine the diversity and representation of the various HIV-1 subtypes throughout the world are under way.

The relationship between viral diversity and disease progression and the impact of diversity on vaccine strategies can be addressed in a lentiviral animal model system. Feline immunodeficiency virus (FIV) was first isolated in 1986 from a cat with symptoms of an immunodeficiency-like syndrome (40) and is emerging as a useful model for understanding immunodeficiency disease. Like HIV-1, FIV can induce immunological abnormalities, including a decline in $CD4^+$ T cells (1, 14, 34), inversion of the $CD4^+/CD8^+$ T-cell ratio (1, 34), and decreased lymphocyte proliferative response to mitogens (2, 48, 53). FIV infection of domestic cats has been found worldwide (16, 17), and related viruses have been isolated from wild members of the family Felidae (i.e., panthers, lions, and bobcats) (3, 36).

The primary translation product of the FIV *env* gene is processed through proteolytic cleavage into the surface protein (approximately 433 amino acids) and the transmembrane protein (approximately 246 amino acids) (54). Pancino and colleagues have defined nine variable regions throughout the FIV Env protein (38). The first two occur in the leader region, which is also the first coding exon of the *rev* gene (41), and are not present in the mature Env protein because of proteolytic processing (54). Variable regions V3 through V6 occur in the surface protein, and variable regions V7 through V9 occur in the transmembrane protein.

The work presented here builds on previous studies of FIV diversity within the domestic cat population (25, 42, 43). Prior to this study, Rigby and colleagues (43) investigated FIV *env* gene diversity and found that the available sequences were nearly equidistant by genetic divergence (with the exception of one isolate from Japan [27]). We evaluated 12 additional FIV sequences obtained in North America and show that they can be divided into three distinct subtypes (designated A, B, and C) by the same criteria used to group HIV-1 strains, with the Japanese isolate serving as the prototype of what we refer to as

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TABLE 1. FIV strains evaluated in this study

Subtype and strain[a]	Origin	No. of cells/μl		Clinical symptom(s)[b]
		CD4	CD8	
A				
USCAlemy00A	Fremont, Calif.	ND[c]	ND	wt loss, OI
USCAhnky00A	El Cerrito, Calif.	ND	ND	wt loss, OI, diarrhea
USCAtt_00A	Oakland, Calif.	108	907	Wt loss, behavior
USCAzepy00A	San Francisco area, Calif.	392	241	None
USCAsam_00A	Albany, Calif.	789	252	Behavior
B				
USOKlgrl00B	Tulsa, Okla.	675	114	None
USILbrny00B	Chicago, Ill.	40	148	None
USMOglwd00B	Kansas City, Mo.	602	345	None
USMAsboy00B	Salem, Mass.	999	1,518	None
USTXmtex00B	Arlington, Tex.	682	1,981	None
C				
CABCpbar00C	Vancouver, British Columbia, Canada	190	519	None
CABCpady00C	Abbotsford, British Columbia, Canada	77	50	wt loss, lethargy

[a] The first two capital letters indicate the country of origin; the next two designate the location within the country (state or province); the four lowercase letters or numbers refer to the name of the cat, virus strain, or isolate; the two numbers represent a specific viral isolate or clone (00 represents the consensus sequence); and the last letter represents the subtype assigned to the virus. For short names, an underline is inserted so that the correct spacing is maintained. This nomenclature evolved from the HIV-1 nomenclature currently being developed (19).

[b] OI, opportunistic infections; behavior, abnormal motor behavior potentially indicative of neurological damage.

[c] ND, not determined.

subtype B. Parallels and distinctions between the patterns of FIV diversity and HIV-1 diversity were also established.

MATERIALS AND METHODS

Viral DNA isolation. Whole-blood samples were obtained in heparinized tubes, and peripheral blood mononuclear cells were separated by Ficoll-Hypaque density centrifugation. For two samples (USCAlemy00A and USCAtt_00A), DNA was extracted from lymph nodes at necropsy. High-molecular-weight DNA was purified by proteinase K digestion and phenol-chloroform extraction (44).

PCR amplification. PCRs were performed in a mixture (100 μl total) containing 10 mM Tris (pH 8.3), 50 mM KCl, 1.7 mM MgCl, 1% dimethyl sulfoxide, 200 μM deoxynucleoside triphosphates, 2.5 U of *Taq* polymerase (Perkin-Elmer Cetus), 10 pmol of each amplimer, and a dilution of cellular DNA corresponding to one FIV provirus (31). Two rounds of PCR (94°C for 45 s, 55°C for 1 min, and 72°C for 2 min 10 s; 30 cycles) in a Perkin-Elmer thermocycler were used to generate a 2,048-nucleotide fragment encompassing the surface and transmembrane portions of the FIV *env* gene. The first-round amplimers were Fenv23 (5′GCGCAAGTAGTGTGGAG ACT) (corresponding to bp 6788 to 6807 of the JapanTM2 genome [27]) and Fenv22 (5′GCTTCATCATTCCTCCTCTT) (corresponding to bp 8836 to 8817 of the JapanTM2 genome). The second-round amplimers were a combination of either Fenv27 (5′GACTGGAATTCGCGCAAGTAGTGTGGAGA CTTCCCCCTTTA) (corresponding to bp 6788 to 6817 of the JapanTM2 genome; with viruses from subtypes B and C) or Fenv31 (5′GACTGGAATTCGCTCAGGTAGTATGGAGA CTTCCACCATT) (corresponding to bp 6784 to 6812 of the Petaluma FIV-14 genome; with viruses from subtype A) and Fenv24 (5′GACTGGGATCCTCATCATTCCTCCTCTTTTT CAGA) (corresponding to bp 8836 to 8810 of the JapanTM2 genome). Although no cloning was performed in the experiments outlined here, restriction enzyme sites (underlined) were included to facilitate cloning of the full-length *env* genes.

DNA sequencing. The 12 new amplified FIV *env* genes were sequenced directly. In order to ensure that each sequence originated from only one FIV proviral genome template, dilutions of the infected peripheral blood mononuclear cell DNA with which less than half of the PCR amplifications resulted in a positive signal were used. The existence of only one FIV genome was further verified by a heteroduplex mobility assay in which the presence of two or more divergent sequences, having a single insertion or deletion, or of point mutations resulting in a mismatch of greater than about 2% would be detected through the formation of slowly migrating heteroduplexes in acrylamide gels (7). Amplified DNA fragments were purified by agarose gel electrophoretic separation and Spin-X centrifuge filtration (Costar). Each fragment was sequenced by using an Applied Biosystems automated sequencer and a dye-deoxy terminator procedure specified by the manufacturer.

Sequence analyses. Overlapping sequences were joined by using the Gel program from the Intelligenetics suite (5). Nucleotide divergence (distance) for pairs of sequences was estimated by using the maximum likelihood method and the DNADIST program from the PHYLIP software package (10). On the basis of the output from DNADIST, phylogenetic trees were constructed by using the FITCH program, and branching order reliability was evaluated by bootstrap analysis (by using the DNABOOT program [10]). K_A, the frequency of amino acid replacement substitutions per replacement site, and K_S, the frequency of substitutions per silent site, were measured by using the method and program developed by Li (21, 22).

Viral sequences. The newly described FIV strains evaluated in this study originated from naturally infected North American pet cats and are described in Table 1. GenBank accession numbers for the V3 to V5 FIV *env* gene sequences are U02392 through U02422. Other FIV sequences used in this study are as follows (GenBank accession numbers and geographic origins are in parentheses): CA.Petaluma clone FIV-14 (M25381) (Petaluma, Calif. [35, 52]); CA.PPR (M36968) (San Diego, Calif. [42]); CA.Dixon (L00608) (northern California [55]);

JapanTM2 (M59418) (Tokyo, Japan [27]); SwissZ1 (X57002) and SwissZ2 (X57001) (Zurich, Switzerland [30]); DutchK1 (M73964) and DutchK32 (M73965) (Amsterdam, the Netherlands [49]); DutchUtr (X60725) (Utrecht, the Netherlands [54]); FranceWo (L06312) (France [28]); ScotUK2 (X69494) (Perth, Scotland [43]); EngUK8 (X69496) (Portsmouth, England [43]); WalesUK14 (X69497) (Colwyn Bay, Wales [43]); Dutch4 (X69498) and Dutch6 (X69499) (Amsterdam, the Netherlands [43]); and ItalyM1 (X69500), ItalyM3 (X69502), and ItalyM4 (X69503) (Pisa, Italy [43]).

For each HIV-1 subtype, the following sequences were used (GenBank or reference numbers are in parentheses): subtype A, U455 (M62320), Z321 (M15896), SF170 (M66535), 1UG06 (M98503), KIG93 (L07082), and D687 (32); subtype B, ALA1 (M38430), BRVA (M21098), SC (M17450), JH32 (M21138), CDC42 (M13137), OYI (M26727), SF2 (K02007), JFL (M31451), RF (M17451), SF162 (M65024), JRCSF (M38429), LAI (K02013), BAL2 (M68894), MN (M17449), NY5NEW (M38431), ADA (M60472), WMJ2 (M12507), 537-1pre and 1058-1pre (6), and HAN (32); subtype C, NOF (L07426), ZAM20 (L03707), D1044 (L07651), D747 (L07653), D757 (07654), D760 (L07655), and IND744, IND766, and IND868 (32); subtype D, ELI (K03454), NDK (M27323), JY1 (J03653), MAL (K03456), UG23 (M98504), Z2Z6 (M22639), Uganda-9 (13), and U44342 (32); and subtype E, TN235 (L03698), TN239 (L03699), TN241 (L03700), TN242 (L03701), TN2432 (L03703), and TN244 (L03704). Intrapatient diversity of HIV-1 was determined with 13 sequences from patient MA (M79342 to M79354) (20), 15 sequences from patient BU (6), 6 sequences from patient PE (6), and 5 sequences from patient JO (6).

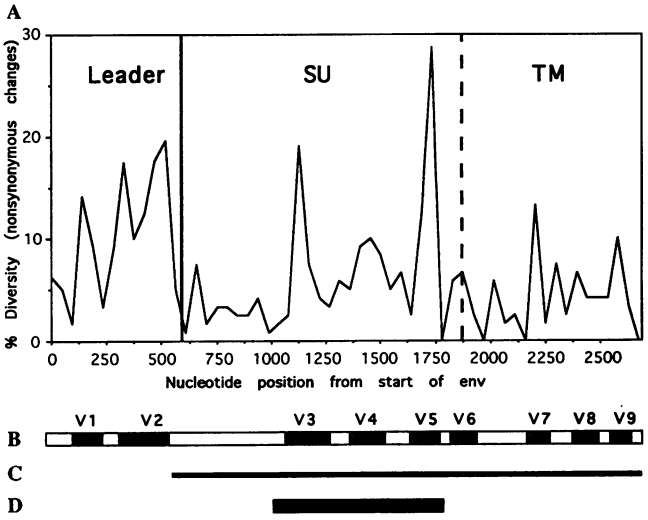

FIG. 1. Variable regions of the FIV Env protein. (A) Variability plot generated from the nucleotide sequence alignment of nine full-length FIV *env* genes (each from a different cat) obtained from GenBank. The sequences used to generate the plot were CA.Petaluma, CA.Dixon, CA.PPR, FranceWo, SwissZ1, SwissZ2, DutchK1, DutchUtr, and JapanTM2. Points on the graph were generated by using a window of 45 nucleotides, and only nonsynonymous changes (those which resulted in an amino acid change) were included. (B) Variable regions of the FIV *env* gene identified by Pancino and colleagues (38). (C) Region of the FIV *env* gene PCR amplified in this study. (D) Region of the *env* gene sequenced and characterized in this study.

RESULTS

The viruses introduced in this study originated from adult domestic cats from the United States and southwestern Canada (Table 1). Data obtained with uninfected adult cats suggest that in general, T-cell counts below 200 cells per μl are abnormally low (15). According to this criterion, 4 of the 12 cats examined had abnormally low $CD4^+$ T-cell levels at the time of sampling. Some of the cats displayed symptoms of immunodeficiency disease (weight loss and opportunistic infections), and two (USCAlemy00A and USCAtt_00A) had died because of complications of immunodeficiency. Clinical symptoms were evident in five cats, including two of the three cats with the lowest $CD4^+$ T-cell numbers (Table 1).

Variable regions within FIV *env* genes. Figure 1A depicts the amino acid variation across the FIV *env* open reading frame determined for the nine full-length *env* genes currently available. Variable regions defined by Pancino and colleagues (38) are indicated (Fig. 1B). To obtain *env* genes directly from feline infected-cell DNA, a nested PCR protocol (31) was used to amplify single FIV proviral templates from peripheral blood mononuclear cells or lymph node cells. The entire coding sequence of the mature Env protein was amplified (Fig. 1C). The nucleotide sequence of the 684-nucleotide region encompassing the most variable region in the *env* gene, V3 through V5, was then determined (Fig. 1D).

To assess the variability of the *env* gene, the 18 sequences available from GenBank were included along with a total of 16 sequences from the subtypes described in Table 1 (Fig. 2). As expected, our results demonstrated a high degree of diversity within the predicted 228-amino-acid region (Fig. 2). This region contains 13 N-linked glycosylation sites, the majority of which were not conserved in all viruses. V5 was the only segment of the *env* gene where length variations were detected. In addition, there were 13 cysteine residues (Fig. 2) conserved in all sequences except ItalyM1, in which three were absent, and ItalyM3, in which one was absent (Fig. 2). All *env* gene segments examined contained intact open reading frames, except the CABCpbar07C sequence, which had one termination codon.

Phylogenetic groupings. An unrooted phylogenetic tree generated for all 34 sequences from V3 to V5 is shown in Fig. 3. This analysis reveals that FIV *env* genes fall within one of three clusters, here designated as envelope sequence subtypes A, B, and C. The A subtype consists of the original isolate from Petaluma plus all other viruses from California and several European countries. The only isolate highly divergent from the isolate from Petaluma in previous studies was the TM2 isolate from Tokyo, Japan (27). TM2 is now viewed as the prototype of what we refer to as subtype B, which includes sequences from the central and eastern United States. Both viruses from subtype C were from southwestern Canada.

In assessing the reliability of the branching order of the phylogenetic tree shown, the data were subjected to bootstrap analysis (9). The bootstrap method recreates the phylogenetic tree multiple times, each time after swapping some characters between sequences, and records the number of times each group of sequences is placed together on the same branch. Branching is considered significant if it occurs in at least 95% of the bootstrap estimates (9). Results from bootstrap analysis of the major branch points are presented in Fig. 3. These analyses demonstrate that sequences within each of the three subtypes are monophyletic (they cluster together) in a significant proportion of the analyses (99 to 100 of 100).

The diversity of the V3-to-V5 region of *env* between FIVs from the same subtype ranged from 2.5 to 15% and did not

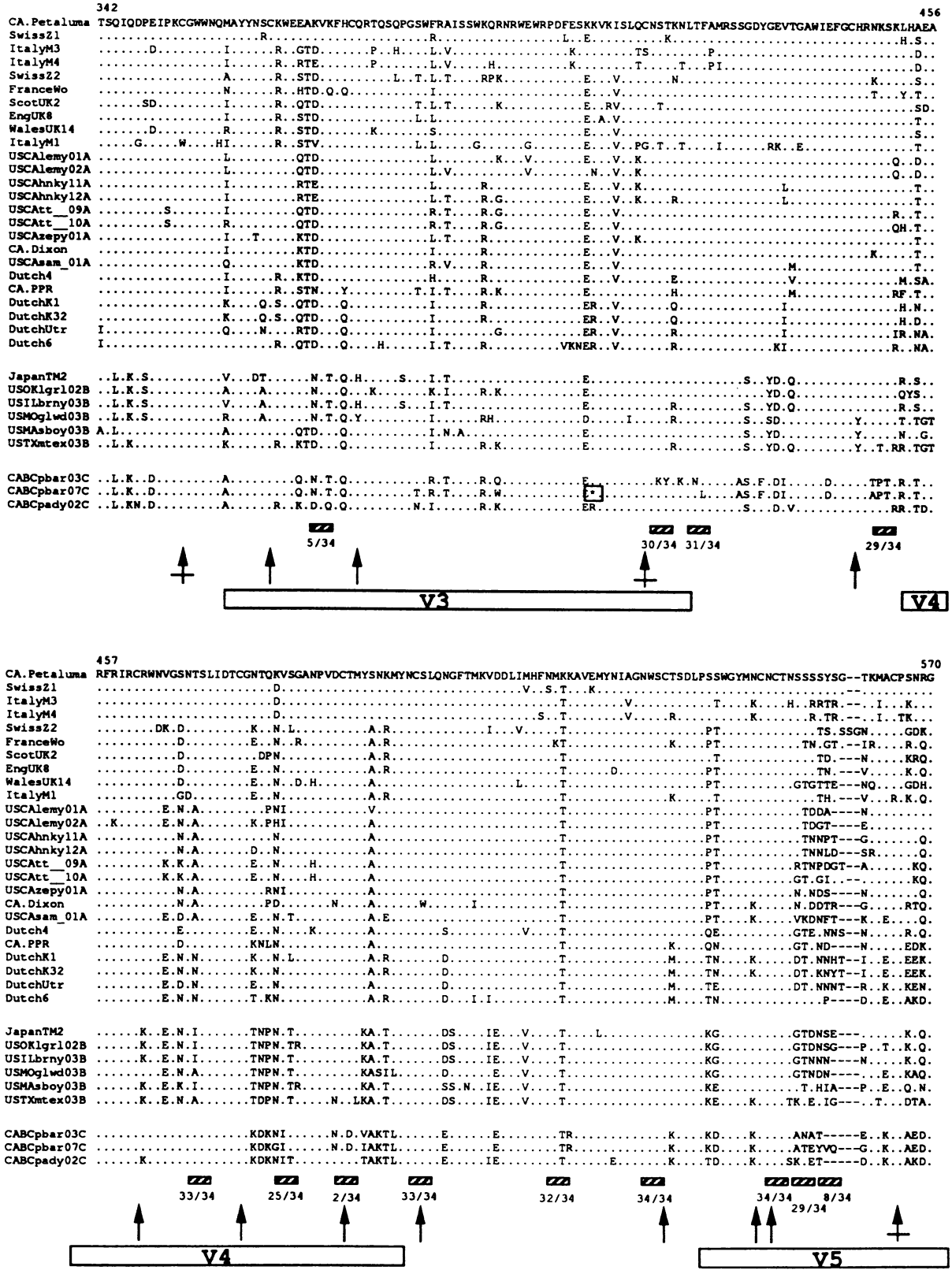

FIG. 2. Deduced amino acid sequences of FIV Env over the region sequenced, beginning 342 amino acids from the start of the leader. Each hatched box indicates the location of an N-linked glycosylation site, and the numbers under each box indicate the ratio of the number of sites present to the total number of sequences examined. Arrows indicate the positions of cysteine residues, and arrows with lines through them indicate cysteine residues not conserved in all sequences (absent from either ItalyM1 alone or both ItalyM1 and ItalyM3). Open boxes indicate the positions of V3 through V5 as defined by Pancino and colleagues (38). Twenty-five sequences (from 21 cats) from subtype A are grouped above six sequences (from 6 cats) from subtype B and three sequences (from 2 cats) from subtype C.

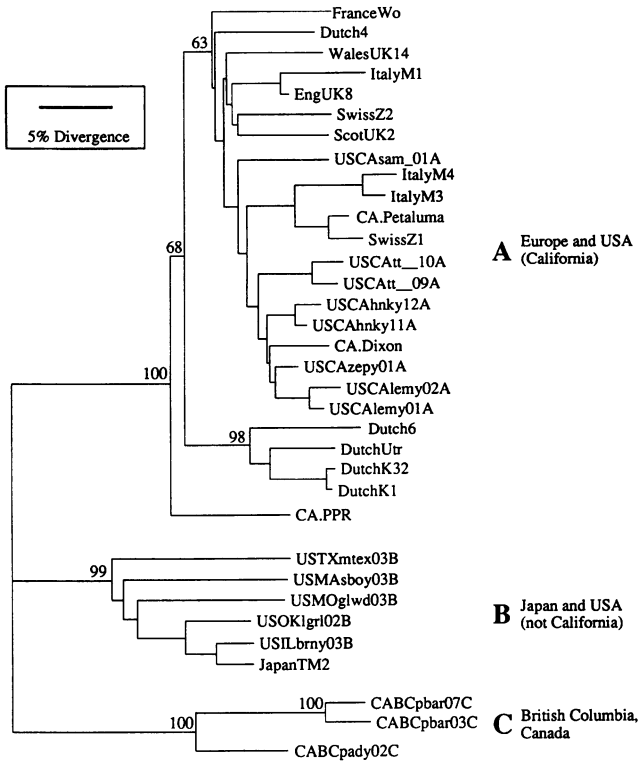

FIG. 3. Phylogenetic analysis of FIV *env* genes. This analysis includes 18 viruses for each of which a 684-nucleotide sequence (V3 through V5) was available from GenBank and 16 viral sequences from this study, including those from each of the subtypes described in Table 1. The diversity within infected cats is represented by two of the most divergent sequences obtained from each cat (USCAtt_00A, USCAhnky00A, USCAlemy00A, and CABCpbar00C). Two sequences obtained from the same cat by Siebelink and colleagues are also presented (DutchK) (49). Only horizontal lines are meaningful in assessing divergence; the distance on the tree which corresponds to 5% divergence is indicated. To evaluate the consistency of the phylogenetic groupings, the data were subjected to bootstrap analysis (9). The number at each branch point indicates the number of times that sequences to the right of the branch of the tree were preserved in 100 bootstrap repetitions.

overlap with viral diversity between different subtypes, which ranged from 17.8 to 26.2% (Table 2). This level of FIV diversity is comparable to the diversity observed within and between HIV-1 subtypes (see Fig. 6).

FIV *env* gene variation within infected animals. In order to assess the diversity of the FIV quasispecies within a cat, multiple viruses were obtained from four cats (Table 3). The two most divergent sequences from each cat are presented in Fig. 2 and 3 (USCAlemy01A and USCAlemy02A,

TABLE 2. Diversity of FIV *env* gene segments between different cats

Subtype	Avg. % diversity of subtype (range):		
	Subtype A	Subtype B	Subtype C
A	9.8 (2.5–15)		
B	21.3 (18.7–24.7)	11.1 (3.3–14.5)	
C	22.9 (20.3–26.2)	20.7 (17.8–22.6)	10.5 (9.9–11)

TABLE 3. Diversity of FIV *env* gene segments within the same cat

Strain	No. of sequences	Avg. % diversity (range)
USCAlemy00A	6	1.1 (0.0–2.1)
USCAhnky00A	4	1.3 (0.9–1.9)
USCAtt_00A	6	1.8 (0.2–2.8)
CABCpbar00C	7	2.5 (1.2–3.7)

USCAhnky11A and USCAhnky12A, USCAtt_09A and USCAtt_10A, and CABCpbar03C and CABCpbar07C). *env* genes obtained from the same cat were usually more closely related than those from different animals. In all, four to seven different *env* genes were obtained from each cat, and the diversity ranged from 0 to 3.7% and averaged 1.1 to 2.5% (Table 3). Intracat diversity of FIV was approximately half of that observed in HIV-1-infected subjects (see Fig. 6).

Relative numbers of amino acid and silent changes. The phylogenetic analysis described above evaluated nucleotide divergence, which includes both amino acid and silent changes. The K_S and K_A have been examined previously in order to understand the selective forces which affect lentiviral evolution (4, 43), including that of FIV (43). In this study, we compared the K_S and K_A values obtained for pairs of FIV *env* gene sequences from different cats (the 684-nucleotide region including V3 to V5) with those obtained for a comparable region (V3 to V5) of HIV-1 *env* gene sequences (Fig. 4), each as a function of nucleotide divergence. K_S values were high (approaching 1) for pairs of FIV sequences when the nucleotide divergence exceeded 15% (Fig. 4A), suggesting that the rate of back mutation was partially counterbalancing forward mutations. Thus, at about 15% overall nucleotide divergence and above (between *env* sequence subtypes), DNA distances are likely to be underestimated. In contrast, the K_S values for HIV-1 sequence pairs were lower; the majority did not exceed 0.5 and the highest was 0.75 (Fig. 4A). If one assumes that the reverse transcriptase error rates for these two viruses are similar, then this result implies that FIV has been in cat populations longer than HIV-1 has been in humans and has therefore accumulated relatively more silent mutations. In contrast, the K_A values obtained for FIV sequences were quite similar to those obtained for HIV-1 sequences. However, when the nucleotide divergence exceeded 15%, the values for HIV-1 sequences were generally higher, indicating somewhat more replacement substitutions per replacement site for HIV-1 than for FIV.

In investigating the evolutionary pressures to which any pair of sequences has been subjected, the values of both K_S (which generally reflects the length of time the two sequences have been evolving apart) and K_A (amino acid replacement sites) are important. In this study, we examined these values as a ratio (K_A/K_S) in order to analyze the different FIV and HIV-1 subtypes (Fig. 5). K_A/K_S ratios of greater than 1 reflect some positive selection for amino acid change (4, 43). In comparison, the K_A/K_S ratio of beta-globin sequences is 0.27, which reflects the high degree of amino acid conservation observed for most eucaryotic genes (22). In examining the K_A/K_S ratios for the FIV subtypes, we found that only seven of the sequence pairs had a K_A/K_S ratio of >1.0, each from a comparison of closely related viruses (<7.5% divergence) (Fig. 5A). Therefore, the majority of sequence pairs studied exhibited no clear evidence for positive selection for amino acid change. Rigby and colleagues described evidence for positive selection for change in FIV *env* when the region analyzed was restricted to the

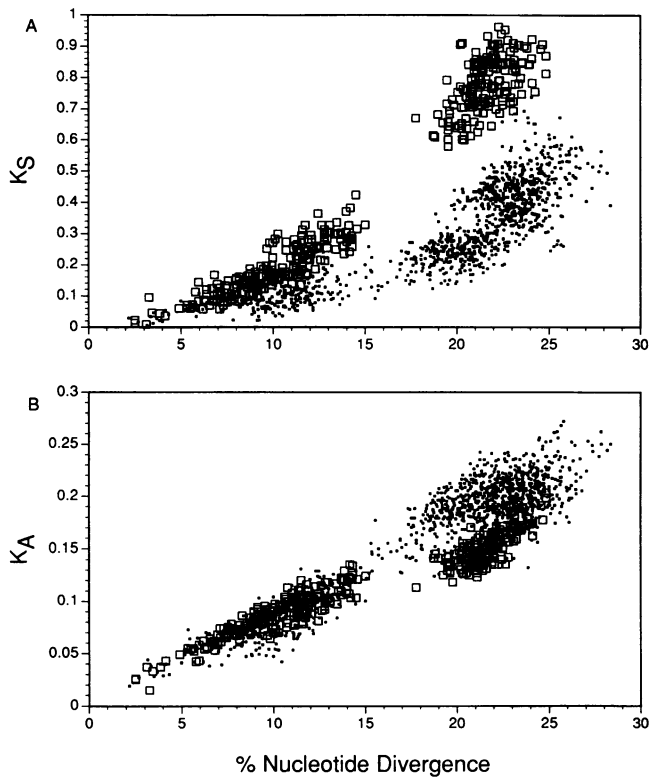

FIG. 4. Silent and amino acid substitutions as functions of total nucleotide divergence. Only viral sequences obtained from epidemiologically unrelated FIV- and HIV-1-infected subjects (within and between subgroups but not intrasubject) were used for these analyses. K_S and K_A were estimated by the method described by Li et al. (21, 22). The K_S (A) and K_A (B) values relative to the amount of nucleotide divergence for both FIV (□) and HIV-1 (•) are presented.

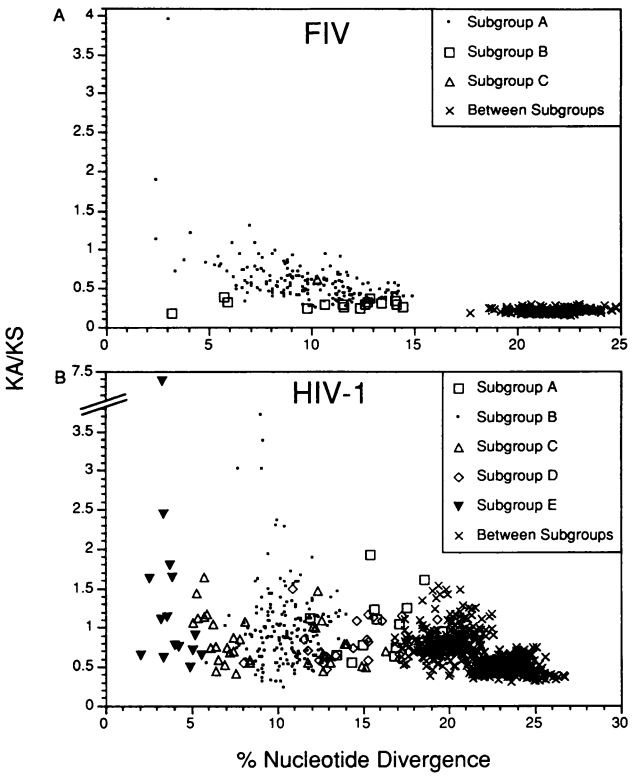

FIG. 5. K_A/K_S ratios and total nucleotide divergence of FIV (A) and HIV-1 (B) *env* gene segments. Only viral sequences obtained from epidemiologically unrelated FIV- and HIV-1-infected subjects (within and between subgroups but not intracat) were used for these analyses. The K_A/K_S values relative to the amount of nucleotide diversity for each pair of sequences within subtypes, as well as for between subtypes, are presented.

variable regions, with V4 having the highest proportion of changes at amino acid replacement sites (43).

The range of K_A/K_S ratios appeared to vary between FIV subtypes (Fig. 5A). When these data were subjected to the Mann-Whitney nonparametric test and a normal approximation, the K_A/K_S ratios were found to be significantly lower ($P < 0.001$) for subtype B than for subtype A. This result suggests that the evolutionary pressures that B subtype viruses were subjected to differ from those affecting A subtype viruses. No subtype-specific pattern for the parallel analysis of HIV-1 was noted (Fig. 5B).

DISCUSSION

FIV sequences previously described were predominately from what we can now define as FIV envelope sequence subtype A, with the exception of the JapanTM2 (27) and the Maryland MD isolates (36) (FIV_{MD} was not examined in this study since only the *pol* sequence is available). The distribution of FIV subtypes exhibited some geographic clustering. Subtype A was found in California and Europe, whereas subtype B was found in Japan and the central and eastern United States. Subtype C was defined by two sequences found in southwestern Canada. Geographic clustering of sequences was also evident within subtype A (e.g., Dutch viruses and California viruses) (Fig. 3), while results obtained within subtype B are more difficult to interpret. For example, the similarity between JapanTM2 and USILbrny03B from Chicago, Ill., is quite striking, considering their geographic distance. We know that this result was not due to contamination since neither the JapanTM2 isolate nor its clone has ever been in our laboratories.

These results obtained for FIV can be compared with those for the simian immunodeficiency virus SIV_{AGM}, a primate lentivirus which can also be divided into phylogenetic subtypes (18, 23). SIV_{AGM} subtypes coincide with different African green monkey species in geographically separate areas of Africa. SIV_{AGM} subtypes exist within clear geographic boundaries, whereas this does not appear to be the case for FIV subtypes, suggesting an earlier entry into and/or the clearly greater mobility of the host (feline) species.

Major interest in FIV derives from its importance as a model for AIDS in humans as well as its importance as a domestic cat pathogen. We therefore sought to determine parallels between the *env* diversity of FIV and that of HIV-1. The region of the HIV-1 *env* gene chosen for comparison included the regions of the surface protein coding sequence from V3 through V5; the region is similar in size, location, and relative level of diversity to the FIV *env* gene fragment analyzed. A total of 47 different HIV-1 *env* sequences obtained from GenBank and representing sequence subtypes A, B, C, D, and E were evaluated (Fig. 6). Parallels in FIV diversity and HIV-1 diversity can be observed among viruses obtained from different subjects of the same subtype (2.5 to 15% for FIV and 2 to 19.5% for HIV-1)

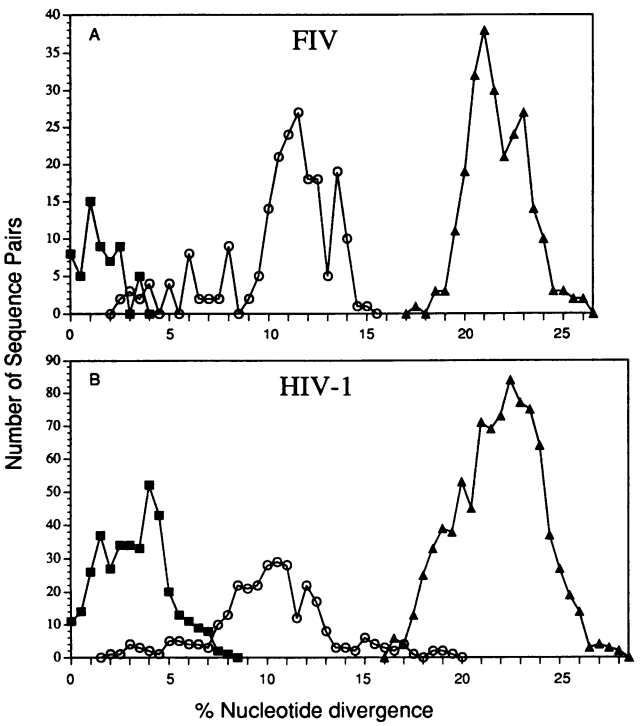

FIG. 6. FIV (A) and HIV-1 (B) diversity within and between infected individuals. Each symbol indicates the number of sequence pairs at a given level of nucleotide divergence. Data within a subject (■) and for subjects infected with viruses from the same (○) or different (▲) subtypes are presented.

or different subtypes (17.8 to 26.2% for FIV and 15.5 to 28% for HIV-1) (compare Fig. 6A and B). The observation that parallels in FIV diversity and HIV-1 diversity exist increases the perceived usefulness of FIV as a lentiviral model system in vaccine development and pathogenesis studies confounded by viral diversity.

Lentiviral diversity within an infected subject was also studied. Multiple viruses representing the pool of variants from four HIV-1-infected people (between 5 and 17 sequences per person) (20, 45) were compared with the pool from four FIV-infected cats (4 to 7 sequences per cat) (Fig. 6). The diversity of sequences of FIV from a cat was approximately half of that observed with sequences of HIV-1 from a patient (mean, 3.5%; range, 0 to 8%). The relatively lower level of FIV diversity within a cat was in concordance with a smaller data set obtained from another investigation (49). However, the level of HIV-1 diversity in humans is in large part a function of the length of time the individual has been infected (39), and the length of time these cats have been infected is unknown.

The analysis of K_S values (proportion of the potential silent changes that have occurred) and K_A values (proportion of the potential amino acid replacement changes that have occurred) provides information as to the selective forces which affect genetic evolution (21, 22). Previous studies have found evidence for positive selection for amino acid change ($K_A > K_S$) within lentiviral *env* genes (4, 33, 43, 46, 51). Previous studies examined the selective forces of FIV diversity by considering the variable and constant regions separately and established that a selection for amino acid change exists within some variable regions of FIV *env* genes, particularly V3 (37) and V4 (43). This selection is likely due to viral mutations resulting in escape from immune recognition. Indeed, biochemical and immunological studies have since verified that the variable regions of Env are targets of the feline immune system (37, 50). The K_A/K_S ratio analysis performed by Rigby et al. utilized the FIV *env* gene sequences available, which were predominately of the A subtype (37, 43). They found even stronger selection for amino acid change within closely related sequences (termed subgroups [43]) of what we now refer to as the A subtype, but they were unable to evaluate differences between subtypes because only one member of the B subtype was available for analysis (JapanTM2).

The additional *env* gene sequences presented here enable us to study the evolutionary differences that exist between FIV subtypes. B subtype viruses were found to have significantly lower K_A/K_S ratios ($P = <0.001$), suggesting a predominant negative or purifying selection rather than a positive selection for change. In a previous study, Shpaer and Mullins (46) examined the selection for amino acid replacement or for silent changes within primate lentiviruses which exhibit diverse pathogenic phenotypes in their respective hosts (8, 11). The K_A/K_S ratios for the A subtype of FIV resembled those for HIV-2 and were about two times lower than those for the pathogenic HIV-1, while the K_A/K_S ratios for B subtype FIV sequences were significantly lower and were similar to those for the relatively nonpathogenic SIV_{AGM} (46, 47). Positive selection results from immune pressure exerted by the infected host (4), and therefore, our data predict a relatively low immune response and a reduced pathogenicity in cats infected by subtype B FIV compared with that in cats infected by subtype A. It is therefore interesting that in this limited study, none of the animals from which subtype B viruses were obtained had evidence of disease, although some of them had low $CD4^+$ cell numbers. Furthermore, these hypotheses can now be tested through experimental analysis of the immunogenic and pathogenic properties of FIVs from different subtypes.

K_A/K_S ratios were also determined for five HIV-1 subtypes (Fig. 5B). We observed that each subtype displayed a broad range of K_A/K_S ratios, with some sequence pairs of a given subtype exhibiting positive selection for amino acid change and others not (ranges: HIV-1 subtype A, 0.64 to 1.92; subtype B, 0.23 to 3.7; subtype C, 0.43 to 1.64; subtype D, 0.55 to 1.5; and subtype E, 0.49 to 2.44). Therefore, in contrast to results for FIV, no subtype-specific patterns were noted for HIV-1.

The finding that FIV exists worldwide in the domestic cat population, the relatively high rate of seroprevalence among domestic cats, and the high K_S values observed for FIV suggest that FIV has been prevalent in cat populations longer than HIV-1 has been in humans. These data support the hypothesis that FIV and its host are more adapted to coexist than are HIV-1 and humans. Nonetheless, the breadth of diversity that exists between FIV sequences suggests that a wide array of challenge strains are available for stringent vaccine protection studies and for the analysis of lentiviral pathogenesis.

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