A Single Mutation within the V3 Envelope Neutralization Domain of Feline Immunodeficiency Virus Determines Its Tropism for CRFK Cells

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Feline immunodeficiency virus (FIV) isolates differ in the ability to replicate in Crandell feline kidney (CRFK) cells. The difference in tropism between two variants of the Dutch isolate FIV-UT113 was studied by using molecular clones which contained the envelope genes of the variants in a background of the FIV-14 Petaluma sequence. Virus produced from clone pPET-113Th replicated in thymocytes, whereas virus from pPET-113Cr propagated in both thymocytes and CRFK cells, thereby reflecting the phenotypes of the parental variants. Exchange of envelope gene fragments showed that a 464-bp surface protein (SU)-encoding fragment encompassing the third variable region (V3) determines CRFK cell tropism. Sequence analysis of the exchanged fragments demonstrated two amino acid changes that led to an increase of the overall charge of the V3 domain: a G→R transition at position 397 and a E→K change at position 407. Mutational analysis of these residues revealed that the E→K shift was responsible for the change in tropism, while the G→R mutation improved the replication kinetics in CRFK cells. Mapping of a tropism determinant for FIV to a region which is also a major neutralization domain is reminiscent of human immunodeficiency virus type 1, in which a similar colocation was found.

Feline immunodeficiency virus (FIV) is a lentivirus that causes a slow progressive degeneration of the immune system that eventually leads to a disease syndrome comparable to AIDS in humans. Lentiviruses are able to replicate in nondividing, terminally differentiated cells (7), and FIV has been found to infect macrophages, astrocytes, B cells, and $CD4^+$ and $CD8^+$ lymphocytes in vitro and in vivo (5, 6, 12, 13, 16). It is routinely isolated by culturing peripheral blood mononuclear cells (PBMC) of infected cats. A few isolates can also replicate in Crandell feline kidney (CRFK) cells (15, 24, 25). Both PBMC and CRFK cells express the putative receptor of FIV, the feline homolog of CD9 (18, 36), indicating that additional cellular factors are required to allow replication (3). A comparable phenomenon has been described for human immunodeficiency type 1 (HIV-1), in which case the presence of the CD4 receptor is not sufficient to allow replication of all HIV-1 isolates in each cell type (14, 17). The in vitro cell tropism of lentivirus isolates appears to have pathogenetic consequences: sheep inoculated with a macrophage-lytic isolate of ovine lentivirus developed a lymphoproliferative disease, whereas animals inoculated with nonlytic isolates remained asymptomatic (22). Molecular clones of simian immunodeficiency virus differing in host cell range have a contrasting pathogenic potential (4). Upon infection with HIV-1, nonsyncytium-inducing macrophage-tropic viruses are isolated; the isolation of syncytium-inducing and T-cell-tropic viruses later in infection heralds progression to AIDS (30, 34). It is unknown whether a similar correlation exists between the cell tropism of FIV and its pathogenic potential.

Transfection of infectious molecular clones of CRFK-tropic virus leads to progeny which infects both PBMC and CRFK cells, whereas virus from non-CRFK-tropic clones infects only PBMC (20, 29). This finding indicates that growth in CRFK cells is blocked at a preintegration step of the infection, and entry would be a likely candidate. The differences between non-CRFK-tropic and CRFK-tropic viruses are unknown, but CRFK tropism has been mapped to the 3′ end of the FIV genome (20). In the present study, we focused on the envelope proteins. By exchanging envelope gene fragments of the Dutch isolate UT113 in a genetic background of the FIV-Petaluma clone FIV-14, we have mapped CRFK tropism on the surface protein (SU) and show that the third variable (V3) region can play a pivotal role in it.

MATERIALS AND METHODS

Viruses and cells. The FIV-14 molecular clone of the Petaluma strain (pFIV-14-Petaluma [25, 26]) and CRFK-HO6 cells, cloned from CRFK cells (8), were used in this study. CRFK-HO6 cells were grown in Dulbecco Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum, 2 µg of Polybrene per ml, and antibiotics (hereafter referred to as DMEM). Thymocytes were isolated from a specific-pathogen-free kitten and maintained in Iscove's medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 400 U of recombinant human interleukin-2 (Eurocetus, The Netherlands) per ml, 2.5 µg of concanavalin A per ml, 2 µg of Polybrene per ml, and antibiotics (hereafter referred to as Iscove's medium).

Construction of pPETΔENV. pFIV-14 was used to construct the vector pPETΔENV (Fig. 1). As the uncharacterized flanking sequences (approximately 8 kb) could interfere with the cloning procedure, e.g., through unidentified restriction enzyme sites, they were removed by PCR; primers matching the 5′ and 3′ ends of the viral genome, CGA*GTCGAC*TGGGATGAGTACTGGAAC (nucleotides [nt] 1 to 19 of the FIV-Petaluma sequence [33]) and TCT*AGATCT*GCGAAGTTCTCGGCCC (nt 9472 to 9456), respectively, were used (SalI and BglII restriction enzyme sites are underlined; non-FIV sequences are in italics). By using the 5′-end primer and an internal primer located in the gag region, PCR was performed for 30 cycles (45 s of denaturation at 94°C, 1 min of annealing at 55°C, and 1 min of elongation at 72°C) in a final volume of 100 µl containing 50 ng of pFIV-14 DNA, 100 ng of each primer, 50 mM KCl, 10 mM Tris (pH 8.3), 2 mM $MgCl_2$, 200 µM deoxynucleoside triphosphate, and 2.5 U Taq polymerase. A similar PCR was performed with the 3′-end primer and a primer located just

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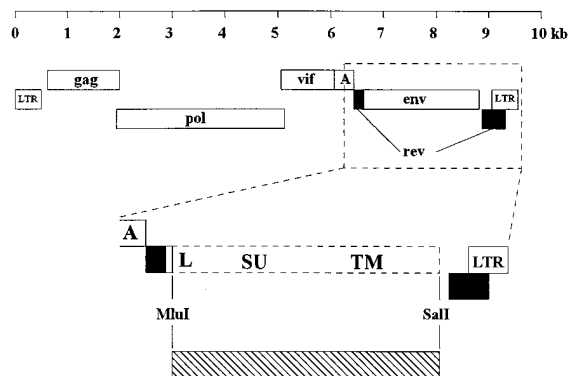
FIG. 1. Construction of molecular clones with different envelope regions. Flanking cellular DNA sequences were removed from the pFIV-14 molecular clone, and the SU-TM coding region was deleted as described in Materials and Methods. The resultant vector, pPETΔENV, was used for the construction of mutant molecular clones by inserting SU-TM regions from other isolates (hatched box). LTR, long terminal repeat.
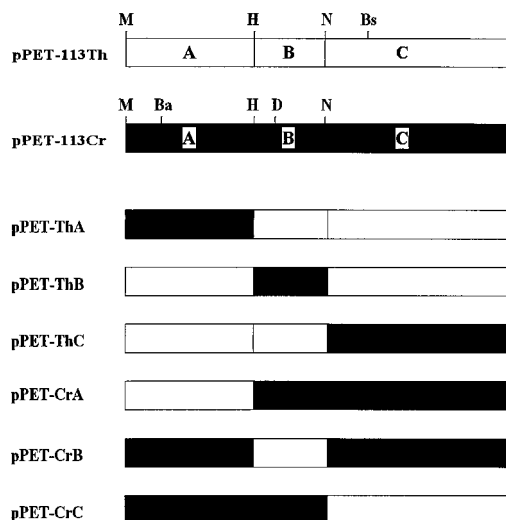

FIG. 2. Schematic representation of the SU-TM regions of the parental and chimeric molecular clones used in this study. The restriction sites used for the exchange of the DNA fragments and for restriction fragment analysis are indicated. M, *Mlu*I; Ba, *Bam*HI; H, *Hin*dIII; D, *Dra*I; N, *Nsi*I; Bs, *Bsu*36I; S, *Sal*I.

downstream from the *env* gene (*TCTGCGGCCGCGTCGAC*AAGGGAGATA CTGTGCTG; nt 8853 to 8886) that introduced a unique *Sal*I site into the genome. The PCR fragment from the 5′ end of the genome was digested with *Sal*I and *Sst*I (located at nt 503 in FIV-Petaluma) and cloned into pUCBM21 (Boehringer Mannheim), resulting in clone pUCPETO-503. Subsequently, the *Sst*I-*Sph*I fragment (nt 504 to 3446) derived from pFIV-14 was inserted into this clone, creating pUCPETO-3446. The PCR fragment from the 3′ end of the genome was digested with *Not*I and *Bgl*II (nt 8858 to 9472) and cloned into pSP73NS (21), yielding pPET8858-9472. By using PCR under the conditions described above with the primers *CCCGGATCC*ATGGCAGAAAGGATTTGC (nt 6264 to 6280; start of the *env* gene) and *TTAGCGGCCGC*ACGCGTCTA AACTTACCTGC (nt 6520 to 6501; introducing a unique *Mlu*I site in the FIV genome), a part of the envelope leader sequence was generated. After digestion with *Bam*HI and *Not*I, it was cloned into pPET8858-9472, creating pPET6264-9472. Then the pFIV-14-derived *Sph*I-*Kpn*I fragment (nt 3447 to 6392) was cloned into pPET6264-9472, creating pPET3447-9472, and finally the *Sal*I-*Sph*I fragment from pUCPETO-3446 was inserted into pPET3447-9472, linearized with *Xho*I and *Sph*I. The resulting clone, pPETΔENV, contained the FIV-Petaluma sequences without part of the envelope leader, the SU sequence, and the transmembrane (TM) sequence (deletion from nt 6520 to 8858). The deletion was flanked by the newly generated unique *Mlu*I and *Sal*I sites. In clone pPETΔENV, the part of the envelope leader sequence overlapping the *rev* open reading frame and its Rev-responsive element (28) had been retained. After insertion of an envelope gene (see below), the leader sequence contained an alanine→valine mutation at the *Mlu*I site. At the *Sal*I site, no known functional open reading frame was interrupted.

Amplification and cloning of envelope sequences. DNA from CRFK cells persistently infected with FIV-UT113, pFIV-14 DNA, and cloned proviral DNA derived from FIV-UT113-infected thymocytes (clone p6.10 [35]) was used for the amplification of envelope sequences. The primers used to amplify the envelope sequence with flanking 5′ *Mlu*I (ACGCGT) and 3′ *Sal*I (GTCGAC) restriction sites were TAG*ACGCGT*AAGATTTTTAAGATACTCTGATG (nt 6512 to 6543) and CTT*GTCGAC*TAAGTCTGAGATACTTCATCATTCCTCC (nt 8861 to 8825), respectively. The PCR mixture was as described above but containing 100 ng of DNA and 1.5 mM MgCl₂. Thirty amplification cycles of 1-min denaturation (94°C), 1-min annealing (55°C), and 2.5-min elongation (72°C) were performed. The products were purified by preparative gel electrophoresis, digested with *Mlu*I and *Sal*I, and cloned into pPETΔENV.

Exchange of envelope fragments. From the molecular clones differing in cell tropism, the *Mlu*I-*Sal*I fragment was cloned into vector pSH, a derivative of pSP73 containing *Mlu*I and *Sal*I cloning sites. Subsequently, the *Mlu*I-*Hin*dIII (fragment A; 828 bp), *Hin*dIII-*Nsi*I (fragment B; 464 bp), and *Nsi*I-*Sal*I (fragment C; 1,025 bp) fragments were exchanged between the clones (Fig. 2). The substitutions were checked by restriction enzyme analysis using unique recognition sites and confirmed by sequence analysis. Finally, the resultant chimeric *Mlu*I-*Sal*I fragments were ligated into vector pPETΔENV.

Site-directed mutagenesis. PCR was used to introduce point mutations in the *Hin*dIII-*Nsi*I fragment (fragment B) of the *env* gene (2). To facilitate the mapping of mutations that induce CRFK tropism, the *Hin*dIII-*Nsi*I fragment was divided into parts of approximately equal size (Fig. 3). The 3′-end fragment B2 was generated by PCR using the primer GCG*AAGCTT*CCATGG*CGCC*TGG ATAGAGTTTGGATG, which introduced a *Kas*I site (*GGCGCC*) into the envelope sequence without changing the amino acid sequence and supplied a *Hin*dIII site (*AAGCTT*) required for cloning. The 3′-primer sequence was identical to the template sequence and was located downstream of the *Nsi*I site. The

PCR mixture contained 100 ng of DNA of either pPET-113Th or pPET-113Cr, 2.5 mM MgCl₂, and the other compounds described above. After amplification as specified above (with 1 min of elongation at 72°C), the fragment was purified, digested with *Hin*dIII and *Nsi*I, and subsequently cloned into *Hin*dIII-*Nsi*I-digested pSH-113Th. The resultant constructs, pSH-113Th-B2Th and pSH-113Th-B2Cr, with a 233-bp deletion in the envelope gene, were checked by sequencing, linearized with *Kas*I and *Hin*dIII, and used for cloning of the (mutagenized) B1 fragments (Fig. 3A). The *Hin*dIII-*Kas*I (B1) fragments of both clones were also generated by PCR, using the same amplification protocol with pPET-113Cr and pPET-113Th, respectively, serving as templates and primers 1 and 4 used in the mutagenesis procedure described below. Amplification products were digested with *Hin*dIII and *Kas*I and cloned into pSH-113Th-B2Th or pSH-113Th-B2Cr. All clones were examined by sequencing, and the *Mlu*I-*Sal*I fragments were inserted into pPETΔENV, thereby creating complete FIV clones with a pPET-113Th background but containing either a B1 or a B2 fragment derived from pPET-113Cr.
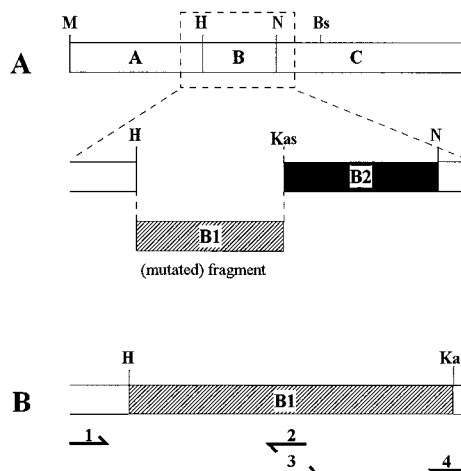

FIG. 3. Site-directed mutagenesis of the *Hin*dIII-*Nsi*I fragment (fragment B) of clone pPET-113Th. (A) Insertion of the *Kas*I-*Nsi*I(B2) fragment to reduce the size of the fragment to be mutagenized; (B) locations of the primers used for PCR-induced mutagenesis of fragment B1. Primer 1, 5′-GGACCTAATCAAA CATGTATGTGG-3′; primer 2, 5′-AAAATCTGGTCTCCATTCCC-3′; primer 3, 5′-GGGAATGGAGACCAGATTTT(GA)AAAGT(GA)AAAGGGTGAAA GTATCG-3′; primer 4, 5′-CTCTATCCA*GGCGCC*CGTTATTTCGCC-3′ (*Kas*I site underlined). Restriction enzyme sites are abbreviated as in Fig. 2.
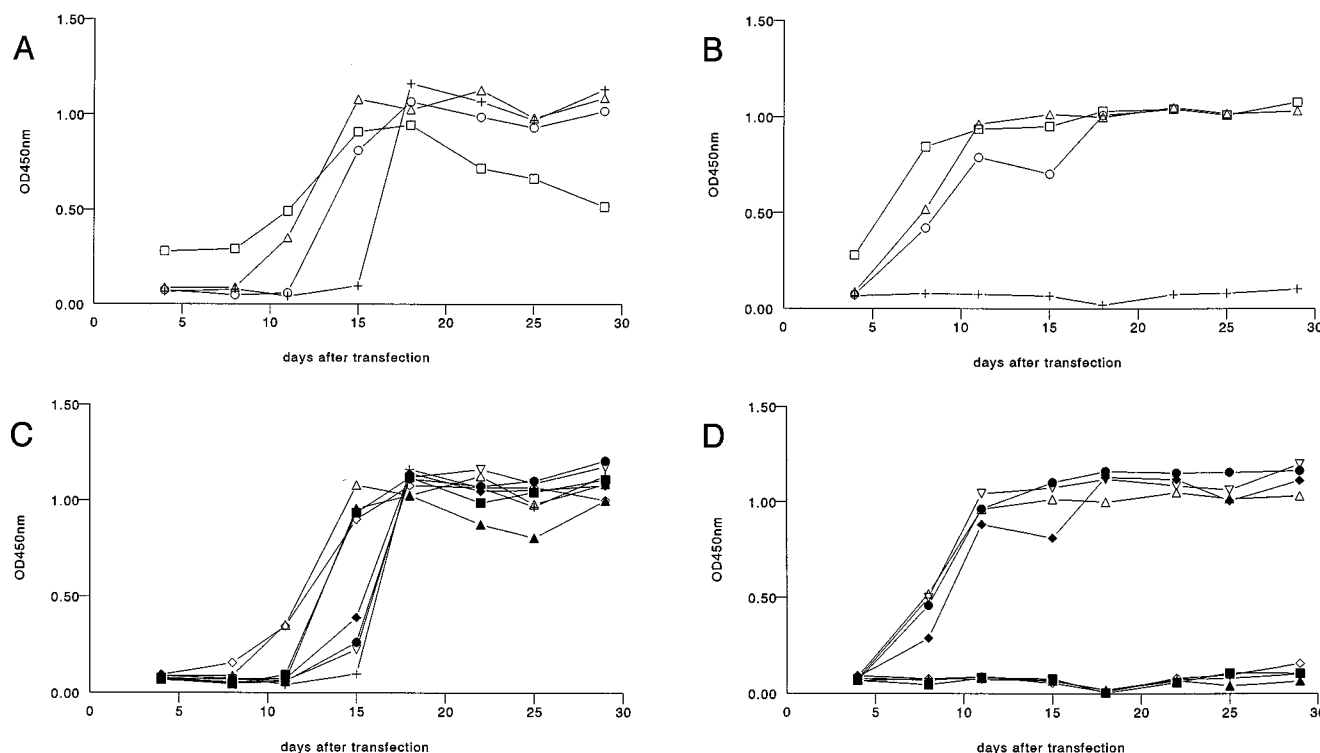
FIG. 4. Replication of viruses in feline thymocytes (A and C) and CRFK-HO6 cells (B and D). Virus replication was monitored by measuring p24 production in the culture supernatants. The replication kinetics of FIV-14 were compared with those from pPET-Pet, pPET-113Th, and pPET-113Cr in thymocytes (A) and CRFK-HO6 cells (B). The replication characteristics of molecular clones chimeric in their envelope genes are shown in panels C (thymocytes) and D (CRFK-HO6 cells). All experiments were repeated at least three times; the results of one representative experiment are shown. □, FIV-14; ○, pPET-Pet; +, PET-113Th; △, pPET-113Cr; ◆, CrA; ■, ThA; ▲, CrB; ●, ThB; ▽, CrC; ◇, ThC. OD450nm, optical density at 450 nm.

The positions of the primers used to introduce point mutations in the B1 fragment are depicted in Fig. 3B. Three sequential amplifications (conditions as specified above) were performed. In the first reaction with primers 1 and 2, either pPET-113Th or pPET-113Cr was used as the template DNA, resulting in amplification products with a G or an R, respectively, at position 397 (see Fig. 5). The degenerated primer 3 was utilized in combination with primer 4 to introduce mutations at positions 407 and 409, using pPET-113Th as a template. Finally, the products of both amplifications were mixed, and an amplification was performed with primers 1 and 4. The mutagenized, amplified fragments were then digested with *Hin*dIII and *Kas*I and cloned into the linearized pSH-113Th-B2Th. Mutant clones were selected after sequence analysis. Finally, *Mlu*I-*Sal*I fragments were transferred from the pSH vectors to the pPETΔENV vector, creating complete molecular FIV clones.

Transfection experiments. Transfection of DNA from molecular clones was performed by the DEAE-dextran method (1). From each clone, 500 ng of DNA mixed with 1 ml of DMEM containing 125 μg of DEAE-dextran was transfected into 10^5 CRFK-HO6 cells by incubation for 4 h at 37°C. The transfection mixture was removed, the cells were incubated for 1 min with 10% dimethyl sulfoxide solution in phosphate-buffered saline (PBS), and then DMEM was added. This medium was replaced 24 h later by 1 ml of Iscove's medium containing 10^5 thymocytes. After 48 h of cocultivation, the thymocytes were removed, washed once with PBS, and resuspended in 200 μl of Iscove's medium. To the remaining CRFK cells, 1 ml of DMEM was added, and both cultures were kept for 30 days at 37°C. Viral replication was monitored at regular intervals by using a p24 enzyme-linked immunosorbent assay.

RESULTS

Reconstruction of the FIV-14 molecular clone results in a replication-competent molecular clone. The molecular clone pFIV-14 served as the genetic background for our chimeric virus constructs. Flanking cellular DNA sequences of unknown function had been deleted from the original clone, and unique *Mlu*I and *Sal*I restriction sites had been introduced to accommodate envelope sequences from other FIV isolates. They were entered into the envelope leader sequence without dis-

turbing the *rev* open reading frame and just downstream of the TGA stop codon of the envelope gene, respectively (Fig. 1). To assess if these modifications affected replication, we constructed a clone (pPET-Pet) which possessed all of the genomic modifications and contained the viral sequences of clone FIV-14 including the envelope gene. DNA from the original (pFIV-14) and modified (pPET-Pet) clones was transfected into CRFK cells, and after cocultivation with thymocytes, virus replication was monitored in both cell types (Fig. 4A and B). The viruses derived from both clones replicated well in both cell types; virus from pPET-Pet showed a somewhat attenuated phenotype, which may due to the removal of enhancing sequences present in the flanking DNA of pFIV-14 or to the introduction of point mutations at the *Mlu*I and *Sal*I sites. The virus originating from the modified clone pPET-Pet was replication competent in both CRFK cells and thymocytes; this finding indicated that the pPETΔENV vector was suitable for the construction of infectious molecular clones chimeric in their envelope sequences.

Tropism of FIV-Petaluma/UT113 envelope chimeras. We constructed two molecular clones that contained envelope gene fragments from thymocyte-adapted FIV-UT113 (pPET-113Th) or from the same isolate adapted to replicate in CRFK cells (pPET-113Cr). The envelope gene fragments encoded part of the leader (L), SU, and TM envelope glycoproteins of FIV-UT113 and were obtained by amplification from DNA. Replication of the viruses originating from the chimeric molecular clones pPET-113Th and pPET-113Cr was monitored in thymocytes and CRFK cells and compared with that of viruses from the parental clones pFIV-14 and pPET-Pet. As illustrated in Fig. 4A, both viruses replicated in thymocytes. In CRFK

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        370            390            410
Cr  QAYYNNCKWERTDVKFQCQRTQSQPGSWIRAISSWKQRNRWEWRPDFKSERVKV
Th  --------------------------------------G---------E------
    ━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━━

        430            450
Cr  SLQCNSTRNLTFAMRSSGDYGEITGAWIEFGCHRNKSIRHNAARFRIRCRWNEG
Th  -----------------------------------------------------
    ━━━━━━━━━━━━━━━━━━                       ━━━━━━━━━━━

        470            490            510
Cr  NNNSLIDTCGETQNVSGANPVDCTMYANKMYNCSLQDGFTMKVDDLIMH
Th  D-----------------------------------------------
    ━━
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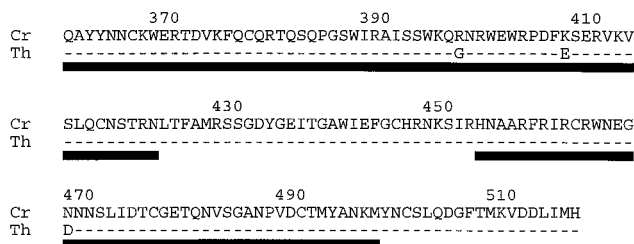
FIG. 5. Comparison of the deduced amino acid sequences of fragment B from pPET-113Th (Th) and pPET-113Cr (Cr) (amino acid numbering according to Verschoor et al. [35]). V3 (amino acids 361 to 422) and V4 (amino acids 452 to 497) domains (27) are indicated by bars.

cells (Fig. 4B), virus from pPET-113Cr replicated whereas virus derived from pPET-113Th did not, revealing the presence of determinants for cell tropism on the viral envelope proteins. The difference in tropism between the viruses from the two clones corresponds to that of the parental variants of FIV-UT113.

CRFK cell tropism is determined by the _Hin_dIII-_Nsi_I envelope gene fragment. To identify the regions of the envelope glycoprotein that influence CRFK tropism, restriction fragments from the envelope gene were exchanged between pPET-113Th and pPET-113Cr (Fig. 2). Virus produced after transfection and cocultivation was then tested for its ability to replicate in thymocytes and CRFK cells. All exchanges resulted in replication-competent viruses, as evidenced by their replication in thymocytes (Fig. 4C). The exchange of the _Hin_dIII-_Nsi_I DNA fragment B between pPET-113Cr and pPET-113Th resulted in a reversal of the capacity of these clones to replicate in CRFK cells (Fig. 4D).

Sequence analysis of the _Hin_dIII-_Nsi_I envelope gene fragment. To investigate which amino acid changes are crucial for the shift in tropism, the _Hin_dIII-_Nsi_I DNA fragments of pPET-113Th and pPET-113Cr were sequenced, and the deduced amino acid sequences were analyzed (Fig. 5). Two mutations were found in the V3 region of the envelope gene (V1 and V2 are located in the leader): a G→R transition at position 397 and a E→K transition at position 407; a third mutation, a D→N transition at position 468, was located in the V4 region of the FIV envelope.

CRFK cell tropism is determined by one amino acid residue located in the V3 domain of the FIV envelope. To facilitate the fine mapping of determinants for CRFK tropism, silent mutations resulting in a _Kas_I restriction enzyme recognition sequence (GGCGCC) were introduced in the middle of the

_Hin_dIII-_Nsi_I fragment (positions 7575 to 7580). The _Hin_dIII-_Kas_I (B1; encompassing the V3 domain) and _Kas_I-_Nsi_I (B2; encompassing the V4 domain) fragments from pPET-113Th and pPET-113Cr were exchanged and introduced into the pPET-113Th background. The B1/B2 chimeras were transfected into CRFK cells and cocultivated with thymocytes, and the derived viruses were analyzed for the ability to replicate in both cell types (Fig. 6). Introduction of the B2 fragment from pPET-113Cr in the pPET-113Th background (a D→N shift at position 468; clone B1Th-B2Cr) did not result in a change of tropism, whereas the B1 fragment exchange did (clone B1Cr-B2Th) (Fig. 6B). This finding implied that CRFK tropism of FIV-UT113 is located in the _Hin_dIII-_Kas_I (B1) fragment of the envelope gene.

PCR-facilitated mutagenesis was used to determine the crucial amino acids within this V3 region. The amino acids at positions 397 (R or G) and 407 (K or E) differing between the CRFK-tropic and non-CRFK-tropic clones, as well as the amino acid at position 409 (K or E), were mutagenized. The rationale for the latter mutation was that CRFK-adapted FIV-Petaluma, in contrast to pPET-113Cr, invariably contains a lysine (K) residue at position 409 while at position 407, either a glutamic acid residue (clone FIV-14) (25, 26) or a lysine (clone 34TF10) (33) can be found. This could signify that the change rather than the exact position is critical. Eight clones with different combinations of the three residues were constructed (Table 1). Together with the parental clones pPET-113Th and pPET-113Cr, they were transfected into CRFK cells, and the mutant viruses were tested for the capacity to replicate in thymocytes and CRFK cells. Only the viruses that contained a lysine residue at position 407 in combination with a glutamic acid residue at position 409 (mutants GKE and RKE) were able to replicate in CRFK cells, although GKE virus replicated less well than the RKE and parental viruses (Fig. 7). All viruses replicating in CRFK cells were checked for additional mutations in the V3 region by using direct sequencing and were found to be identical to the clones used for transfection. No shift to CRFK tropism was found when a lysine residue was inserted at position 409 (mutants GEK and REK; data not shown). After transfection with mutants RKK and GKK, no virus replication was observed in thymocytes and CRFK cells, although transient expression of viral proteins could be detected. Radioimmunoprecipitation analysis of the envelope proteins produced from clones RKK and GKK did not explain their lack of replication capacity, as these proteins did not differ in electrophoretic mobility or expression level from the replication competent viruses (data not shown).
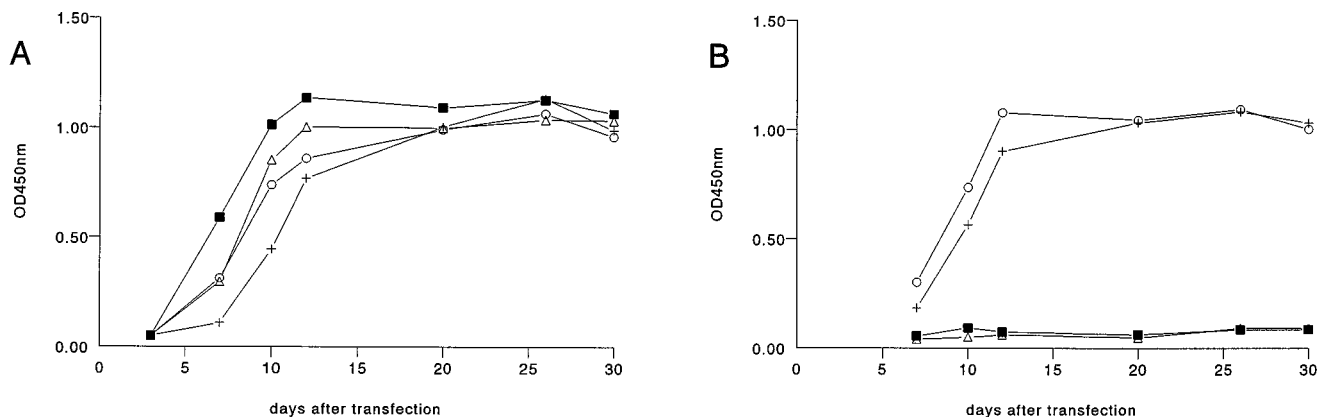

FIG. 6. Replication of fragment B1/B2 mutants in thymocytes (A) and CRFK-HO6 cells (B). Virus replication was monitored by measuring p24 production in the culture supernatants. +, B1Cr-B2Cr; △, B1Th-B2Th; ○, B1Cr-B2Th; ■, B1Th-B2Cr. OD450 nm, optical density at 450 nm.

TABLE 1. Amino acid sequences and overall charges of V3 regions of parental and mutant molecular clones

Clone	V3 Amino acid sequence (positions 377–417)[a]	Charge
pPET-113Th	CQRTQSQPGSWIRAISSWKQGNRWEWRPDFESERVKVSLQC	3+
pPET-113CR	--------------------R---------K----------	6+
GKK	--------------------G---------K-K--------	7+
GKE	--------------------G---------K-E--------	5+
GEK	--------------------G---------E-K--------	5+
GEE	--------------------G---------E-E--------	3+
RKK	--------------------R---------K-K--------	8+
RKE	--------------------R---------K-E--------	6+
REK	--------------------R---------E-K--------	6+
REE	--------------------R---------E-E--------	4+

[a] Amino acid residues differing between the clones are indicated.

DISCUSSION

The objective of this study was to locate determinants for cell tropism of FIV. We focused on the envelope proteins, using two variants of the Dutch isolate FIV-UT113 which differ in CRFK cell tropism. On the basis of the infectious FIV-14 (Petaluma) molecular clone, a system that allowed the cloning of envelope genes from different FIV isolates was developed. Thus, chimeric viruses which contain and encode the SU and TM envelope proteins of the two tropism variants of FIV-UT113 were created. Their phenotypes correspond to those of the parental viruses: virus produced from clone pPET-113Th replicates in thymocytes, while virus from pPET-113Cr replicates in both thymocytes and CRFK cells. Although determinants elsewhere in the genome cannot be excluded, our data show that the envelope gene of FIV contains at least one determinant for CRFK tropism. This observation is in agreement with that of Kohmoto et al. (20), who have recently shown that tropism for CRFK cells is determined by the 3′ end of the genome of FIV-Petaluma. By exchanging fragments of the envelope gene of the two variants, we identified the determinant for CRFK tropism in the V3 region of the SU protein.

This region is also a major neutralization domain as determined on CRFK cells (11, 24). The presence of a neutralization domain and a tropism determinant in the same region implies that V3 is important for the entry of FIV into CRFK cells. The nature of the interaction of the V3 region with proteins of the cytoplasmic membrane is unknown. Recently, feline CD9 has been proposed as the receptor of FIV (36); this molecule is expressed on both CRFK and T cells, and infection of both cell types can be blocked by a monoclonal antibody to CD9 (3, 18). The interaction of V3 with the host cell may involve a secondary receptor rather than the CD9 protein. If this assumption is valid, the V3 region of FIV would resemble that of HIV-1: this region also contains a tropism determinant and a major neutralization domain and interacts with secondary receptor proteins different from the CD4 receptor (for a review, see reference 23).

Fine mapping of the CRFK tropism determinant showed that the mutation from a glutamic acid to a lysine (E→K; charge change of +2) at position 407 was sufficient to convert a non-CRFK-tropic virus into a CRFK-tropic virus. A concurrent transition of a glycine to an arginine (G→R; additional charge change of +1) at position 397 creates a CRFK-tropic virus (RKE) with wild-type replication characteristics overcoming the reduced replication kinetics of the CRFK-tropic virus with the single E→K mutation (GKE). Mutant REK grows in thymocytes but is unable to replicate in CRFK cells; it contains the E→K mutation at position 409 found in the CRFK-tropic FIV-Petaluma clone FIV-14, and its putative V3 loop (27) has a charge identical to that of the CRFK-tropic UT113 virus (6+). A further increase of the overall charge in the V3 region (GKK and RKK) results in viruses unable to replicate CRFK cells and thymocytes. In contrast, the V3 region of the Petaluma infectious molecular clone 34TF10 (33) also contains the RKK mutations and an overall charge identical to that of the RKK-UT113 mutant. The phenotypes of these mutants mimicking the Petaluma V3 domain but accommodated in an FIV-113 envelope background indicate that the effect of a specific amino acid change and concurrent charge shift is restrained by its context within the isolate-specific envelope proteins.
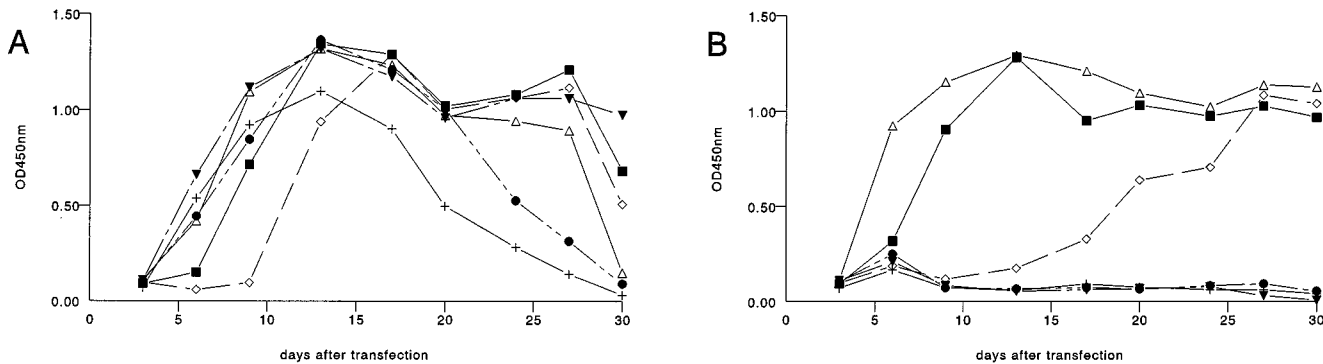

FIG. 7. Replication of pPET-113Th point mutants in thymocytes (A) and CRFK-HO6 cells (B). Virus replication was monitored by measuring the p24 production in the culture supernatants. +, pPET-113Th; △, pPET-113Cr; ■, RKE; ●, REE; ◇, GKE; ▼, GEE. OD450 nm, optical density at 450 nm.

Our observations with the GKE and RKE mutants show that single amino acid changes in the V3 domain can influence the tropism. These changes result in an overall positive charge shift of the V3 region. In HIV-1 also, positive charge shifts in the V3 loop of gp120 have been correlated with an alteration in the host cell range (10, 31); in addition, single mutations in the V3 region can change the virus's tropism (9, 32).

Functions carried out by the V3 region of FIV are surprisingly similar to those in HIV-1. Although no sequence similarity between the viruses exists in this region, the region in each virus encompasses an immunodominant neutralization domain and an important determinant for cell tropism (in contrast with the V3 region of simian immunodeficiency virus [19]). Tropism and interaction of the virus with the immune system are crucial to pathogenesis. The similarity between FIV and HIV-1 underlines the importance of FIV infection of cats as an animal model for human AIDS.

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