Isolation and sequencing of infectious clones of feline foamy virus and a human/feline foamy virus Env chimera

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Full-length DNAs of the Coleman and S7801 strains (pSKY3.0, pSKY5.0) of infectious feline foamy viruses (FFVs) were cloned and sequenced. Parental viruses, designated SKY3.0 and SKY5.0, were secreted following transfection of Crandell feline kidney (CRFK) cells. Production of the rescued parental viruses was enhanced in the presence of trichostatin A. Amino acid sequence similarities between FFV and human foamy virus (HFV) are extremely low for the envelope protein and capsid antigen, as predicted from the two clones. However, a chimeric FFV clone was constructed with the HFV Env substituted for the FFV Env. The chimeric virus (HFFV, SKY4.0) was able to infect and replicate in CRFK cells as well as in peripheral blood mononuclear cells of cats in vivo. Consequently, the chimeric HFFV may be useful for the creation of FV vectors for gene transfer strategies.

The genomic DNA of feline foamy virus (FFV) contains structural genes designated gag, pol and env, as well as the auxiliary gene bel (Helps & Harbour, 1997). These genes are located between two long terminal repeats (LTRs). Although this type of genome organization is common for foamy viruses (FVs) from several species of mammals, there are remarkable dissimilarities between FVs and other retroviruses in their mode of replication (Yu et al., 1999). For example, interactions between group-specific antigen (Gag) and envelope protein (Env) are essential for the assembly and budding of infectious FV particles (Pietschmann et al., 1999), whereas the Gag protein alone is sufficient to allow budding in cells infected with retroviruses other than FVs (Linial, 1999). Reports indicate that it is difficult to produce high titre pseudotyped FV bearing glycoproteins from murine leukaemia virus Env or vesicular stomatitis virus G proteins (Lindemann et al., 1997; Hill et al., 1999). These observations indicate that the interaction between FV Gag and Env of different species of origin may be of interest when evaluating FVs as vector candidates for gene transfer. We report the isolation and sequencing of FFV clones SKY3.0 and SKY5.0. Furthermore, we show that a chimeric FFV clone (HFFV) bearing Env and a part of the trans-activator (Tas) from human foamy virus (HFV) can infect and replicate in HFV-susceptible cells.

Coleman, S7801 and Sammy-1 strains of FFV were grown as described previously (Hatama et al., 2001). Crandell feline kidney (CRFK) cells were infected with each FFV strain, and total DNA was extracted using the Total DNA Extraction kit (Stratagene) according to the manufacturer’s instructions. Preliminary experiments revealed that the extracted DNA contained a number of circular forms of unintegrated viral DNA which contained single LTRs. To construct the infectious clones, pSKY1.0 and pSKY2.0, the extracted DNA was amplified by PCR using two primer pairs, 5′ agctgatgtcc-tggaggatgtgcttccc 3′ (nt 10239–10267 in the FUV genome; accession no. Y08851) and 5′ gcactctcctcctgcagttgcatgttgacata 3′ (nt 10267–10293), and 5′ ggaatggaatgccataaaacacactaca 3′ (nt 6357–6385) and 5′ ctagggctctactgagggaggtg 3′ (nt 1415–1443). Amplified DNAs were cloned into pCR2.1 (Invitrogen) to give two clones designated as 5′LTR-gag-pol (pSKY1.0) and env-bel-3′LTR (pSKY2.0). To construct the full-length Coleman clone, pSKY3.0, the Sall and SpeI double-digested fragment of pSKY2.0 was inserted into pSKY1.0 after digestion of the plasmid with the same enzymes. A similar procedure was used to construct the full-length S7801 clone, pSKY5.0. The DNA sequences of the FFV clones, pSKY3.0 (accession no. AB052796) and pSKY5.0 (accession no. AB052797) were determined using a DSQ-2000L DNA sequencer (Shimazu Co.). The Coleman and S7801 clones were 11694 and 11660 bp in length. Comparisons of the putative Gag, Pol, Env, Bel I and Bel II amino acid sequences between either SKY3.0 or SKY5.0 and FUV (Winkler et al., 1997) gave
similarity estimates of 95.9, 96.9, 82.4, 89.8 and 94.2%, and 96.9, 95.4, 83.3, 94.3 and 93.6%, respectively. Gag, Pol, Env, Bel I and Bel II amino acid sequence similarities between either SKY3.0 or SKY5.0 and HFV (Loehelt et al., 1991) were 34.7, 60.1, 42.3, 17.6 and 31.8%, and 34.4, 59.7, 43.0, 46.2 and 36.4%, respectively. To investigate whether HFV Env and FFV Gag proteins can assemble together, we constructed an HFV Env (Tas) chimeric clone. The infectious clone of HFV-N (pHS007) has been described previously (Adachi et al., 1995). DNA representing the full-length *env* and a part of *bel I* of HFV-N (3229 bp) was amplified by PCR using primers 5' aaatgaataaagcgcatgagg 3' (nt 7185–7205) and 5' taacagctcagggtcagtatc 3' (nt 10444–10465). The amplified fragment was inserted into the *SalI* and *MroI* double-digested pSKY3.0 after blunting the ends (HFFV, pSKY4.0; accession no. AB052798) (Fig. 1A). The fragment representing the HFV *env*
PCR-positive; ‡ amplified by PCR. Detection of PCR products was performed with agarose gel electrophoresis (Fig. 1A). FFV genome. The full-length FFV Coleman clone (pSKY3.0) was constructed from clones pSKY1.0 (5′-half) and pSKY2.0 (3′-half). The entire env region from an infectious HFV-N clone was amplified by PCR. The chimeric FFV with HFV env plasmid (pSKY4.0) was constructed by inserting an approximately 3.2 kbp fragment (env and a part of bel I) from pHFV-N into the Sall (nt 6394)–MboI (nt 9622) blunted site of pSKY3.0. The predicted initiation and termination sites of the env and bel I ORFs, and both sites of the insert are depicted. Exact insertion points are represented by arrows in the nucleic acid sequences. Amino acid residues and numbers are also shown. IP, internal promoter. (B) Morphological alteration of cells infected with chimeric virus. Typical syncytia were formed in CRFK cells infected with FFV Coleman strain (b) and SKY3.0 (c), and BHK-21 cells infected with HFV-N (e) and SKY4.0 (f). The respective mock-infected cells are shown in (a) and (d). The plaque morphologies are observed at ×200 magnification after staining with May–Grunwald–Giemsa. (C) Replication kinetics of parental viruses from infectious DNA clones. The FFV infectious clones, pSKY1.0 plus pSKY2.0, pSKY3.0 and pSKY5.0 were transfected into CRFK cells and the HFFV chimeric clone, pSKY4.0, was also transfected into BHK-21 cells. At 30 h post-transfection, the appropriate parental viruses were secreted into the cell-free supernatants and cultured with 1 ml of complete medium. CRFK cells infected with either the Coleman strain of FFV or SKY3.0 showed cytocidal effects with the formation of large syncytia (Fig. 1B b, c). HFV-N as well as SKY4.0 also produced marked cytopathic effects (CPE) in BHK-21 cells (Fig. 1B e, f). A non-cytopathic, persistent infection was established following four passages of SKY3.0- or SKY4.0-infected cells with uninfected cells. SKY3.0 and SKY4.0 DNAs were detected by PCR in the persistently infected cells, as well as in acutely infected CRFK and BHK-21 cells (data not shown). These data indicate that the cloned FFV and HFFV DNAs can persist in the cells after repeated passage. Transfection of pCR2.1 as a negative control did not produce CPE in CRFK or BHK-21 (Fig. 1B a, d). The virus titre was measured daily for 7 days using a CPE assay with end-points reported as TCID₅₀ (Ikeda et al., 1997). Cell-free supernatants from pSKY3.0-, pSKY4.0- and pSKY5.0-transfected CRFK cells yielded approximately 10² TCID₅₀ after 30 h. After infection with supernatants from transfected cells, titres of SKY3.0 and -5.0 parental viruses at day 3 were 10⁶ and 10³ TCID₅₀/ml, respectively (Fig. 1C). Titres of SKY1.0 plus SKY2.0 viruses were lower (approximately 10⁴ at day 4) than when full-length recombinant clones were used. The titre of SKY4.0 recombinant virus (HFFV) was approximately 10⁵ TCID₅₀/ml at day 3 (Fig. 1C).

The CPE of FFV, HFFV and HFV-N were examined on CRFK, BHK-21, Gin-1, HeLa, HL60 and PC12 cells (Table 1). HFFV derived from pSKY4.0 induced syncytia formation in and a part of bel I was substituted for the equivalent region of FFV (aa 1–129 of HFV Tas, I was converted to T at aa 130, and fused with aa 131–209 of FFV Tas) (Fig. 1A). FV plasmid DNA (20 μg/ml) was transfected into FFV or HFV permissive cells (5 × 10⁵) using 5 μg of lipofectin (Gibco) in the presence of 10⁻³ M of trichostatin A (TA), as described previously (Hatama et al., 2001). Cells (1 × 10⁵) were infected with 0.1 ml of the cell-free supernatants and cultured with 1 ml of complete medium. CRFK cells infected with either the Coleman strain of FFV or SKY3.0 showed cytocidal effects with the formation of large syncytia (Fig. 1B b, c). HFV-N as well as SKY4.0 also produced marked cytopathic effects (CPE) in BHK-21 cells (Fig. 1B e, f). A non-cytopathic, persistent infection was established following four passages of SKY3.0- or SKY4.0-infected cells with uninfected cells. SKY3.0 and SKY4.0 DNAs were detected by PCR in the persistently infected cells, as well as in acutely infected CRFK and BHK-21 cells (data not shown). These data indicate that the cloned FFV and HFFV DNAs can persist in the cells after repeated passage. Transfection of pCR2.1 as a negative control did not produce CPE in CRFK or BHK-21 (Fig. 1B a, d). The virus titre was measured daily for 7 days using a CPE assay with end-points reported as TCID₅₀ (Ikeda et al., 1997). Cell-free supernatants from pSKY3.0-, pSKY4.0- and pSKY5.0-transfected CRFK cells yielded approximately 10² TCID₅₀ after 30 h. After infection with supernatants from transfected cells, titres of SKY3.0 and -5.0 parental viruses at day 3 were 10⁶ and 10³ TCID₅₀/ml, respectively (Fig. 1C). Titres of SKY1.0 plus SKY2.0 viruses were lower (approximately 10⁴ at day 4) than when full-length recombinant clones were used. The titre of SKY4.0 recombinant virus (HFFV) was approximately 10⁵ TCID₅₀/ml at day 3 (Fig. 1C).

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Table 1. Susceptibility of cell lines to infectious foamy virus clones

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<tr>
<th>Cell line</th>
<th>Species/tissue</th>
<th>Syncytia formation</th>
<th>PCR</th>
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<td></td>
<td></td>
<td>FFC</td>
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<tr>
<td>CRFK</td>
<td>Feline kidney</td>
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<td>BHK-21</td>
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<td>HL60</td>
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<td>PC12</td>
<td>Rat adrenal medulla pheochromocytoma</td>
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and a part of bel I was substituted for the equivalent region of FFV (aa 1–129 of HFV Tas, I was converted to T at aa 130, and fused with aa 131–209 of FFV Tas) (Fig. 1A). FV plasmid DNA (20 μg/ml) was transfected into FFV or HFV permissive cells (5 × 10⁵) using 5 μg of lipofectin (Gibco) in the presence of 10⁻³ M of trichostatin A (TA), as described previously (Hatama et al., 2001).
HFV-susceptible BHK-21 cells, but not in the Gin-1, HeLa or HL60 human, or the CRFK feline, cell lines (Table 1). FFV and HFV derived from pSKY3.0 and pHFV-N caused the expected syncytia formation in CRFK and BHK-21 cells. CRFK, BHK-21, Gin-1, HeLa and HL60 cells contained FFV-, HFFV- and HFV-specific gag DNA that was detectable by PCR (Table 1). These results indicate that the pattern of CPE produced by the chimeric viruses in various mammalian cell lines is consistent with the species of origin of the \textit{env} gene, and that FV receptors are all similarly distributed on different mammalian cell lines.

To investigate the infectivity of the FFV (SKY3.0) and HFFV (SKY4.0) molecular clones \textit{in vivo}, FVs were injected into four specific-pathogen-free male cats (cats A and B, SKY3.0; cats C and D, SKY4.0). Cats were infected by the intraperitoneal route with $10^{15}$ TCID$_{50}$ of FFV from supernatants of infected cells and sera were obtained at 30 days post-infection. Antibodies against FFV and HFFV were detected in the FV-infected cats by immunoblotting (Otake et al., 1994). Serum from SKY3.0-infected cat B was used to probe lysates of Coleman strain- or SKY3.0-infected CRFK cells and SKY4.0- or HFV-N-infected BHK-21 cells (Fig. 2A). Bands of the 48 and 53 kDa Gag proteins were observed in infected cells, but not in mock-infected or HFV-N-infected cells (Fig. 2A). Serum from mock-infected cat #1 did not react with the Gag proteins (Fig. 2A). Similar results were obtained using sera from cats A, C and D (data not shown). His-tagged Gag protein was expressed in \textit{E. coli} and also used as a target antigen for immunoblotting with the cat sera. The recombinant FFV gag-
expressing plasmid pFFV/01 was constructed by inserting the blunted Xhol–Sall fragment (nt 1525–3696) into the SalI-digested and blunted pQE02 plasmid (Qiagen). The Histagged Gag protein was extracted and purified using Nitrilotriacetic acid (NTA) column chromatography (Qiagen). Sera containing anti-Gag polyclonal antibody were obtained from rabbits hyperimmunized with purified His-tagged Gag. Sera from cats B and C reacted with the 53.5 kDa recombinant Gag protein (Fig. 2B), while pre-immune sera were non-reactive (data not shown). Ni-NTA alkaline phosphatase conjugates and anti-Gag rabbit serum reacted with the Gag protein (Fig. 2B), while pre-immune sera were non-reactive (data not shown). Ni-NTA alkaline phosphatase conjugates and anti-Gag rabbit serum reacted with the Gag protein bands. Both pre-infection and infected sera from cats A and D, infected with SKY3.0 and SKY4.0, respectively, gave results comparable to those obtained with sera from cats B and C (data not shown). To investigate whether viral genomes were present in the peripheral blood mononuclear cells (PBMC) of infected cats, total DNA was extracted from PBMC at 30 days post-infection and analysed by nested PCR. Three pairs of primers, 5′ aggacctgaaagacatg 3′ (nt 1732–1748) and 5′ ttgttgagtagctgcccttg 3′ (nt 2528–2545) for FFV gag, 5′ ttagctccctaggagagg 3′ (nt 8384–8400) for FFV env and 5′ aagcttgttagccgaagttgttgagatctgcggctgtagaaatcctcgacgcc 3′ (nt 9416–9435) and 5′ ttgaagaaaatcctcgacgcc 3′ (nt 9727–9746) for chimeric bel I, were used for the first-round PCR. Three additional pairs, 5′ aggacctgaaagacatg 3′ (nt 1732–1748) and 5′ aagcttgttagccgaagttgttgagatctgcggctgtagaaatcctcgacgcc 3′ (nt 9416–9435) and 5′ ttgaagaaaatcctcgacgcc 3′ (nt 9727–9746) for chimeric bel I, were used for the first-round PCR. Three additional pairs, 5′ aggacctgaaagacatg 3′ (nt 1732–1748) and 5′ aagcttgttagccgaagttgttgagatctgcggctgtagaaatcctcgacgcc 3′ (nt 9416–9435) and 5′ ttgaagaaaatcctcgacgcc 3′ (nt 9727–9746) for chimeric bel I, were used for the second-round PCR. FFV gag-specific DNA was detected in SKY3.0- and SKY4.0-infected cats (Fig. 2C). FFV env- and chimeric bel I-specific DNAs were detected only in cats infected with SKY3.0 and SKY4.0, respectively (Fig. 2C). The PBMCs from the negative control, mock-infected cat #1, yielded no bands, while the pSKY3.0 and pSKY4.0 positive control gave the expected FV-specific bands (Fig. 2C). Therefore, these results suggest that SKY3.0 and SKY4.0 are replication competent in vivo, and the viral DNA is harboured in the PBMC for at least 30 days after infection.

Our data suggested the feasibility of using chimeric FFV genomes to produce replication-competent FFV particles. Low titres of cell-free, secreted FV are a practical disadvantage for exploitation of FV clones as vectors (Zemb et al., 2000). The use of TA as a supplement in the transfection procedure (Hatama et al., 2001), along with the use of CRFK cells containing β-gal reporter plasmids as a sensitive detection system (Yu & Linial, 1993), gives extracellular titres of approximately 10⁶ f.f.u./ml. The titres may be greater than those obtained previously for HFV by Yu & Linial (1993). The functional domains of HFV Tas, the promoter-targeting domain, the nuclear localization signal and the transcription activation domain, have been reported to be located within the central and carboxyl-terminal region of the Tas protein (He et al., 1996). The chimeric Tas of HFFV contains aa 1–129 from the amino-terminal region of HFV Tas and aa 131–209 from the carboxyl-terminal region of FFV Tas. Although amino acid sequence similarity was low between HFV and FFV Tas, the chimeric tas gene was functional in generating a viral protein which is able to activate the FFV promoter. These results indicate that the major functional domains of the chimeric Tas are probably localized in the central to carboxyl-terminal region. Thus, HFFV is likely to be a very useful tool for a DNA delivery system. In previous studies, attempts to pseudotype HFV using Env from other retroviruses did not result in secretion of particles (Lindemann et al., 1997; Pietschmann et al., 1999). However, we found that the HFFV chimera can produce progeny viruses. Thus, it is suggested that HFV Env can interact with the FFV Gag protein to give a productive replication cycle. Although several cell lines are susceptible to HFFV in vitro (Hooks & Gibbs, 1975), our results indicate that HFV-(Russell et al., 1996) as well as FFV-based vectors may be suitable for gene transfer.

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References


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