Infectivity of lion and puma lentiviruses for domestic cats

Sue VandeWoude, Stephen J. O’Brien† and Edward A. Hoover

Department of Pathology, College of Veterinary Medicine, Colorado State University, Fort Collins, CO 80523, USA

Infection of domestic cats with feline immunodeficiency virus (FIV) causes progressive immunological deterioration similar to that caused by human immunodeficiency virus (HIV). Lentiviruses related to but phylogenetically distinct from FIV have been detected in several non-domestic feline species. Serological cross-reactivity of these viruses raises the question as to whether inter-species transmission may occur. To address this issue, we asked whether lion lentivirus (FIV-Ple) or two strains of puma lentivirus (FIV-Pco) could replicate or cause disease in domestic cats. We found that domestic cats inoculated with FIV-Ple developed persistent cell-associated viraemia, transient cell-free viraemia and antiviral antibody. Clinical disease was not detected throughout a 6 month observation period. Two of four cats inoculated with FIV-Pco developed cell-associated viraemia, seroconverted and exhibited transient lymphadenopathy. No changes in white blood cell parameters or other haematological abnormalities were detected in any of the infected cats. Virus-specific RNA was detected in co-cultivated lymphocytes of all infected cats by RT-PCR. These findings reveal that non-domestic cat lentiviruses are infectious for domestic cats and can establish persistent infection in the absence of disease.

Introduction

Viruses related to human immunodeficiency virus (HIV) infect several animal species. Feline immunodeficiency virus (FIV), the lentivirus of domestic cats, and simian immunodeficiency virus (SIV) induce immunodeficiency syndromes similar to HIV and thus are important models for AIDS research. Simian lentiviruses do not cause an AIDS-like illness in their natural host species, likely due to the long period of host adaptation to the virus which has occurred in the African monkey species (Hirsch et al., 1989; Murphey-Corb et al., 1986). By contrast, when inoculated into Asian primates, SIV causes immunodeficiency syndrome in these related, yet non-adapted hosts (Letvin & King, 1990; Murphey-Corb et al., 1986).

Naturally occurring FIV in domestic cats is typically marked by a long clinical latency period during which a gradual immunological deterioration occurs. Like HIV, FIV is lymphotropic and induces alterations in circulating T-helper and T-suppressor cell populations. Naturally infected animals ultimately exhibit a wide variety of opportunistic infections and haematological abnormalities similar to those described in humans with AIDS (Pedersen, 1993; Sparger, 1993; Sparger et al., 1989).

Serological surveys of non-domestic feline species have determined that a significant number of these animals have antibodies cross-reactive with FIV antigens (Barr et al., 1989; Brown et al., 1993a, b, 1994; Carpenter et al., 1996; Hofmann-Lehmann et al., 1996; Letcher & O’Conner, 1991; Lutz et al., 1992; Olmsted et al., 1992; Spencer et al., 1992). Genetic characterization of puma (Puma concolor), lion (Panthera leo), pallas cat (Felis manul) and bobcat (Lynx rufus) isolates has determined that these viruses are distinct from each other and related to FIV (Barr et al., 1995; Brown et al., 1994; Carpenter et al., 1996; Langley, 1996; Olmsted et al., 1992). The pathogenic nature of these diseases in their natural hosts has not been fully characterized. While one report associated lymphoma in a captive lion with an FIV-like lentivirus, other analyses have not correlated specific disease expression with seropositivity (Brown et al., 1994; Hofmann-Lehmann et al., 1996; Olmsted et al., 1992; Poli et al., 1995). Analogous to the primate biology of SIV, certain populations of African lions appear to be endemically infected with FIV-Ple, whereas Asian lions are seronegative (Brown et al., 1994).
In this study we examined the potential for cross-species transmission of the feline viruses to determine whether infection could occur and whether disease would result based on the precedents observed in the primate lentivirus system.

**Methods**

**Animals.** Nine weanling cats were obtained from the Colorado State University specific-pathogen-free cat colony. Animals were housed by inoculation group in gang rooms. Animals were weighed and blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 weeks post-inoculation. Physical examinations were also performed at these times and on days 1–6 and 10. Lymph node and bone marrow biopsies were performed at week 24.

**Virus stocks.** FIV-Ple was an expanded stock of RT- and EM-positive supernatant obtained from a seropositive Serengeti lion (Ple458; Brown et al., 1994). Three cats were each inoculated with 2 × 10^6 viable FIV-Ple-infected cat PBMC with 0.7 ml supernatant and 2 × 10^6 viable FIV-Ple-infected 3201 cells with 1.3 ml of supernatant. Two strains of FIV-Pco, PLV-14 and PCLV, were used in this study. PLV-14 originated from an infected Florida Panther (Langlely et al., 1994; Olmsted et al., 1996). PBMC from this animal were cultured in vitro in puma PBMC; this supernatant was expanded in 3201 feline lymphoma cells (VandeWoude et al., 1996). PCLV cell-free and cell-associated virus was obtained from a persistently infected 3201 cell line originally inoculated with PBMC from a seropositive British Columbian puma. Two cats were inoculated with each of these strains of FIV-Pco. Each cat was inoculated with 1 × 10^7 viable FIV-Pco-infected 3201 cells and 1.0 ml of supernatant. The two FIV-Pco isolates used represent the two clades of puma lentivirus (PLV-14, clade A; PCLV, clade B) which have been distinguished by phylogenetic analysis of a conserved region of the pol gene (Carpenter et al., 1996; S. VandeWoude, S. J. O’Brien, J. P. Slattery, K. Langelier, W. D. Hardy & E. A. Hoover, unpublished results). Two control cats received 0.5 × 10^7 naive 3201 cells and 2.5 × 10^6 naive cat PBMC in 1.0 ml of naive 3201 supernatant. Infection of inocula was confirmed by RT assay. All inoculations were performed intravenously while cats were sedated with ketamine anesthesia.

**Co-cultivation/RT assay.** Previous studies indicated that all three viruses readily infected 3201 cells, and cell-free virus could be detected by RT assay (VandeWoude et al., 1996). Whole blood was collected from inoculated cats at the time-points indicated above. Plasma and PBMC were isolated using standard methodologies (Quackenbush et al., 1990). Plasma (0.5 ml) and 10^6 PBMC were co-cultivated with 10^6 3201 cells. Supernatant was collected bi-weekly for 4 weeks. The final collection was analysed for magnesium-dependent RT activity using a modified microtitre 3H-P detection assay (Goldstein et al., 1990; Willey et al., 1988). Results were recorded using autoradiography and β emissions of samples bound to DEAE-81 paper.

**Flow cytometry.** Feline T lymphocyte immunophenotype labelling was performed with monoclonal antibodies to feline CD4 and CD8 (O’Reilly & Hoover, 1993) as described by Dean et al. (1991). Lymphocyte subset percentages were analysed with a Coulter EPICS Profile II flow cytometer. Total CD4+ and CD8+ cell numbers were calculated from subset percentages, total leukocyte numbers and lymphocyte percentages of total leukocytes.

**Western blot.** Crude FIV-Ple and FIV-Pco viral protein preparations were electrophoresed on 12% polyacrylamide and transferred to nitrocellulose, similar to previously described methods (Diehl et al., 1996; VandeWoude et al., 1996). Dilutions (1:60 to 1:100) of sera from inoculated cats were incubated with the appropriate immunoblot strips and Western blot assays performed. FIV-infected cat sera and naive cat sera served as positive and negative controls.

**Lymph node, bone marrow biopsy and culture.** Prescapular lymph nodes were collected aseptically while animals were anaesthetized with ketamine–acepromazine–halothane anaesthesia. Tissue was homogenized through a fine sieve, washed twice in PBS and resuspended at a concentration of 10^6 cells/ml. Bone marrow was aspirated with an 18 gauge bone marrow biopsy needle from the femur at the time of surgery. Bone marrow mononuclear cells were purified on a Percoll gradient and washed three times in PBS. Bone marrow and lymph node cells (10^6 of each) were cultured and assayed for RT activity as described above.

**RT–PCR.** RNA was isolated from 200 μl of supernatant from week 24 PBMC co-cultivations using QiAmp Blood Kit reagents (Qiagen), as per product directions, and eluted into RNase-free water. The RNA was recovered by ethanol precipitation and resuspended in 23 μl of water, half of which was used for cDNA synthesis using a commercial kit (cDNA Cycle Kit, Invitrogen). One-third of the cDNA reaction was used as a substrate in hot start PCR and the following 35 cycle programme: 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min. Nested primers were used to further amplify FIV-Pco isolates; these amplifications were performed at 55 °C annealing temperatures. The following virus-specific primers were designed from a conserved region of the pol gene for each virus and were synthesized commercially ( Gibco BRL Custom Primers).

PLV-14-specific primers (Olmsted et al., 1992): 1258F (bp 2430), 5’ GAAGCATTAACAGAATAGTAG 3’; 1260R (bp 3007), 5’ GTTCCTTGGTTGTAATTTATCTTC 3’; 1261R (bp 2990), 5’ ATCTTCAGAGGTTCCTAAAATTCCCA 3’; 1259 (bp 2460), 5’ GAAGGAAAGGTAAAAAGACACAGATC 3’.

PCLV-specific primers (VandeWoude et al., 1996): C24F (bp 2449), 5’ GAAGATTAGAAAGAGGGAAA 3’; C530R (bp 2975), 5’ AATCTCCACACAGTAAATAAA 3’; C146F (bp 2572), 5’ AATGCGAAGACTGAGAAAAGG 3’; C359R (bp 2803), 5’ AGGGGCTTAAATATCCATCCT 3’.

FIV-Ple-specific primers (VandeWoude et al., 1996): L5–23F (bp 2526), 5’ AAAAAAGAATCAGGAAAAATA 3’; L5–345R (bp 2868), 5’ ATGGGATGTTTTATCCTAAA 3’.

Each reaction contained 1.5 mM MgCl₂, 150 μM dNTPs, 8 U of AmpliTaq DNA polymerase (Perkin-Elmer) and each primer at 1 μM.

**Results**

**Physical examination**

Mean weight of FIV-Ple-inoculated cats and FIV-Pco-inoculated cats did not vary significantly from the two control cats during the course of the study. Cats P1 and Pc2 had palpable peripheral lymphadenopathy at week 6 which lasted for 6–18 weeks (Fig. 1). No other clinical abnormalities were noted throughout the course of the study.

**Plasma viraemia**

See Figs 1 and 2. All three FIV-Ple-inoculated cats had detectable plasma viraemia at week 1. Cat L2 was also viraemic at week 2; cat L1 had a positive plasma culture at week 12. None of the FIV-Pco-infected cats had positive plasma cultures at any time-point.
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Fig. 1. Summary of plasma viraemia, cell-associated viraemia, seroconversion and peripheral lymphadenopathy detected in domestic cats inoculated with FIV-Ple or FIV-Pco over a 24 week period. Week PI, weeks post-inoculation; C1 and 2, control cats; L1, 2 and 3, FIV-Ple-inoculated cats; Pc1 and 2, British Columbia FIV-Pco-inoculated cats; P1 and 2, PLV-14-inoculated cats; nd, not done. The methodology is detailed in the text.

Fig. 2. Magnesium-dependent RT assay of FIV-Ple- and FIV-Pco-inoculated cat plasma and cell co-culture supernatant. Supernatant samples were collected after 4 weeks of co-cultivation of week 24 post-inoculation PBMC and processed as described in the text. The figure shows autoradiography (overnight exposure) of radiolabelled RT-generated product bound to DEAE-81 filter paper; exposure indicates a positive RT reaction. C1 and 2, control cats; L1, 2 and 3, FIV-Ple-inoculated cats; P1 and 2, PLV-14-inoculated cats; Pc1 and 2, British Columbia origin FIV-Pco-inoculated cats. -, RT-negative control (naive 3201 supernatant); +, positive-RT control (supernatant from a 3201 cell line persistently infected with PcLV); F, domestic cat FIV Crandell cell supernatant.

Table 1. Presence of FIV-Ple or FIV-Pco in prescapular lymph node or bone marrow 6 months post-inoculation

Methods are detailed in the text. C1 and 2, control cats; L1, 2 and 3, cats inoculated with FIV-Ple; Pc1 and 2, cats inoculated with British Columbia isolate of FIV-Pco; P1 and 2, cats inoculated with PLV-14 isolate of FIV-Pco. Ab indicates seropositive or seronegative status; LN indicates presence of virus in 10^6 lymph node cells as detected by co-cultivation; BM indicates presence of virus in 10^6 bone marrow mononuclear cells as detected by co-cultivation.

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Differential cell counts

White blood cell counts remained within the normal range (5.5–20.0 x 10^3 cells/ml) for all animals at all time-points except for occasional sporadic measurements. Lymphocyte counts similarly remained within normal range for cats (1.5–7.0 x 10^3 cells/ml) for most time-points.

Flow cytometry

CD4, CD8 and total lymphocyte counts of FIV-Ple- or FIV-Pco-infected cats did not differ significantly from control animals. CD4 counts remained above 400/ml for all animals at all time-points, and no downward trends were seen in infected cats. The CD4/CD8 ratio remained above 2 for nearly all time-points in all animals; when the value approached 2 or below it was due to an increase in CD8 cells rather than a decline in CD4 cells. In contrast, studies with FIV have documented a significant decline in CD4 count (less than 400/ml) and inversion in CD4/CD8 ratio within 4–8 weeks post-inoculation, (Bendinelli et al., 1995; Dua et al., 1994; O’Neil et al., 1995; M. H. Myles & E. A. Hoover, unpublished results) though attenuated strains of FIV-Fca may be less capable of altering lymphocyte subset kinetics (Sparger et al., 1994).

Western blot

Seroconversion was detected in all three FIV-Ple-inoculated cats by week 3 (Figs 1 and 3). Cats Pc2 and P1 seroconverted by week 6 and week 12, consecutively, as determined by Western blot analysis.

RT–PCR

FIV-Ple primers amplified an approximately 340 bp fragment in supernatant from PBMC cultures of cats L1, L2 and L3 but not from controls or naive supernatant. PLV-14-specific

PBMC viraemia

See Figs 1 and 2. All three FIV-Ple-inoculated cats had detectable cell-associated viraemia at nearly all time-points. Cats Pc2 and P1 were positive by week 4 and remained positive throughout the monitoring period. Cat Pc1 had a marginally elevated RT activity at week 6; cat P2 was culture negative throughout the study. Both control cats had negative plasma and PBMC cultures at all time-points.

Lymph node and bone marrow

Virus was detected in bone marrow samples from cats L1 and L3 and prescapular lymph node samples from cats L2, Pc2 and P1 (Table 1).

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Fig. 3. Week 16 post-inoculation Western blot results. Immunoblotting was done as indicated in the text. +, positive control (domestic cat FIV immune sera from chronically infected cat); −, negative control (naive cat sera); PcLV 1 and 2, British Columbia origin FIV-Pco-inoculated cats; PLV 1 and 2, PLV-14-inoculated cats; C1 and 2, control cats; FIV-Ple 1, 2 and 3, FIV-Ple-inoculated cats. Arrows indicate major Gag proteins recognized; p46 and p26 for PLV and P26 and P17 for FIV-Ple.

Fig. 4. Week 24 PBMC co-culture supernatant RT–PCR. Supernatant samples were identical to those shown in Fig. 3. The methodology for performing RT–PCR is described in text. PCR reactions were run on 1% agarose gel and stained with ethidium bromide. FIV-Ple primers were used to amplify supernatant from FIV-Ple-inoculated cats (L1, 2 and 3), control cats (C1 and 2), no DNA (H2O) and a positive control (L; FIV-Ple pol plasmid clone). PLV-14 primers were used to amplify supernatant from PLV-14-inoculated cats (P1 and 2), control cats, no DNA and a positive control (P; PLV-14-inoculated 3201 cells). PcLV primers were used to amplify supernatant from PcLV-inoculated cats (Pc1 and 2), control cats, no DNA and a positive control (Pc; PcLV pol plasmid clone). M, DNA ladder. Arrows indicate approximate migration of 500 and 200 bp fragments. PLV-14 and PcLV reactions were second round nested reaction products. RT–PCR was also done on naive 3201 supernatant with each set of primers; all these samples were negative for DNA by agarose gel electrophoresis and ethidium staining (not shown).

primers amplified an approximately 525 bp product from cat P1 after nested PCR with primers 1259 and 1261. PcLV-specific primers amplified an approximately 230 bp fragment after nested RT–PCR in Pc2 but not other supernatants (Fig. 4). These sizes are consistent with the predicted amplification products from these primers. Previous work (VandeWoude et al., 1996) has shown that FIV-Ple and FIV-Pco primers do not cross-amplify each other in this type of RT–PCR reaction, even when annealing temperatures of 37 °C are used. This RT–PCR confirmed that viruses cultivated were consistent with the input virus for each seropositive cat.

Discussion

Previous attempts at infection of domestic cats with FIV-Ple have been unsuccessful (Lutz et al., 1992) whereas in this study three of three inoculated cats became persistently infected. Our ability to detect infection in these animals may have been enhanced by an in vitro propagation/RT assay system, although infection was also detectable by seroconversion and PCR assays. The FIV-Ple isolate used may have been better able to replicate in domestic cats than other field isolates. This is suggested by the observation that the FIV-Ple used in this study was only one of five isolates that could be co-cultivated in lion PBMC (Brown et al., 1994) and was readily expanded in cat PBMC and a feline lymphoma cell line (VandeWoude et al., 1996). Because this FIV-Ple could be cultured in vitro, the input inoculum was likely higher than that previously used. In addition, it is possible that amplification of this virus in domestic cat PBMC and a domestic cat cell line allowed selection of a strain of FIV-Ple that can readily infect domestic cats in vivo.

Olmssted et al. (1992) found that one domestic cat inoculated with WBMC from a seropositive puma became persistently infected but did not show obvious clinical signs of disease. Additionally, analysis of lentiviral sequences obtained from PBMC of 44 seropositive pumas identified one animal (a captive puma from Peru) that was apparently infected with a strain of domestic cat FIV (Carpenter et al., 1996). Two of four cats inoculated with FIV-Pco in the current study became...
infected, one each with PcLV and PLV-14. Although serological cross-reactivity between PcLV and FIV-C has been reported (Langelier et al., 1995) PcLV did not appear to be any more infectious or pathogenic for domestic cats than the Florida Panther strain of FIV-Pco. This is consistent with the finding that this virus was classified phylogenetically as a clade B puma lentivirus similar to other FIV-Pco strains from this geographical region (VandeWoude et al., 1996).

All three cats inoculated with FIV-Ple-infected cells and cell-free virus seroconverted rapidly and developed transient viraemia in 1 week. In contrast, only two of four cats inoculated with FIV-Pco seroconverted. These two FIV-Pco-infected animals had persistent cell-associated virus 2–4 weeks post-inoculation, lymphadenopathy, virus in prescapular lymph node cells, but no detectable plasma viraemia. These findings suggest a subtle difference between the course of infection of these strains of FIV-Pco and FIV-Ple in domestic cats.

Although domestic cats became infected with both FIV-Pco and FIV-Ple, none of the animals developed T cell deficits and clinical symptoms were mild (lymphadenopathy in the two FIV-Pco-infected cats). The viruses may have become attenuated by propagation in cell culture; PcLV in particular had been passaged in 3201 cells for several months prior to inoculation. Due to the sero-relatedness of PcLV and FIV-C (K. M. Langelier, W. M. Hardy & K. E. Atkinson, unpublished results) further examination of the potential infectivity of uncultured isolates may be warranted. A second explanation for lack of virulence is based on phylogenetic analysis of these viruses. The degree of pol gene sequence divergence between highly conserved regions of FIV-Pco, FIV-Ple and FIV is 20–30% (Brown et al., 1994; Carpenter et al., 1996; Omlsted et al., 1992). Such diversity suggests these viruses have been present in their respective feline hosts for many generations, perhaps since divergence of the felid species from a common feline ancestor (Carpenter et al., 1996; Omlsted et al., 1992). During this time-span, the viruses may have become less virulent due to immunological adaptation and host selection (Carpenter & O’Brien, 1995).

Epidemiological studies have shown that women infected with the relatively non-virulent HIV-2 are less susceptible than are uninfected cohorts to infection with HIV-1 (Travers et al., 1995). Chickens infected with herpesvirus of turkeys are protected from challenge with the virulent Marek’s disease herpesvirus of chickens (Bülow, 1977; Okazaki et al., 1970; Purchase et al., 1972). Similarly, attenuated measles virus will replicate in dogs without adverse effects and then protect these dogs against canine distemper virus challenge (Brown et al., 1972; Greene, 1990; Wilson et al., 1976). By analogy, it is possible that non-domestic cat lentivirus could protect cats against FIV challenge, thereby providing a model system for inter-species lentiviral vaccination.

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References


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