Fetal Feline Immunodeficiency Virus Is Prevalent and Occult

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The feline model of human acquired immune deficiency syndrome, feline immunodeficiency virus (FIV), was used to model maternal-to-fetal human immunodeficiency virus type 1 (HIV-1) transmission. Fetuses were collected from cats infected with clade A FIV_Petaluma (FIV-A-Pet) and clade C FIV_PaddyGammer (FIV-C-Pgmr). Virus was detected by DNA polymerase chain reaction (PCR) and a quantitative RNA assay that used substitutional PCR. FIV in tissue sections was assayed by DNA and RNA in situ hybridization and immunohistochemistry. Viral DNA was detected in 67% and 92% of fetuses with FIV-A-Pet and FIV-C-Pgmr, respectively. A discordance was observed between placental and fetal FIV-A-Pet localization. FIV sequestration in fetal tissues was common without detectable virus in blood (occult infection). Viral RNA was not detected in amniotic fluid or fetal plasma. In situ assays demonstrated FIV DNA but neither RNA nor antigens in placentas and fetuses. If mother-to-offspring FIV infection reflects vertical HIV-1 transmission, fetal virus exposure is common and may be occult at term.

Nearly 1.5 million children are infected with human immunodeficiency virus type 1 (HIV-1), and, at the current rate, one-third of them will die within a year [1]. In 2000, ∼600,000 children were newly infected, virtually all of them the result of mother-to-child transmission [1]. Chemotherapy has dramatically reduced the rate of vertical HIV-1 transmission in industrialized nations, but its impact in developing countries has been limited by cost, availability, and other factors [2]. Even where chemoprophylaxis is readily available, maternal compliance rates are frequently poor [3], and some drugs are potentially toxic to the developing fetus [4–7].

The discovery of alternative modes of intervention will be accelerated by a clearer understanding of the basic mechanisms operating in mother-to-child HIV-1 transmission. Information is particularly lacking regarding antenatal transmission. Because in utero interventions in humans are necessarily limited, animal models are needed to fully explore this mode of transmission. Feline immunodeficiency virus (FIV) infection of domestic cats causes disease that is virtually identical to human AIDS [8]. HIV-1 and FIV share a basic lentiviral genomic organization, structure, life cycle, and range of cell targets. Of importance, certain FIV isolates are readily transmissible in utero, even in chronically infected animals [9–11]. This distinguishes FIV from the simian immunodeficiency virus (SIV)/macaque model of AIDS, which appears best suited to studies of virus transmission during and after birth [12–14]. Thus, FIV affords a unique opportunity to characterize spontaneous maternal-to-fetal lentivirus transmission in a relevant animal model.

Here, we report maternal-to-fetal transmission of clade A FIV_Petaluma (FIV-A-Pet) and clade C FIV_PaddyGammer (FIV-C-Pgmr). These 2 FIV isolates were chosen to complement studies elsewhere that demonstrated intrauterine transmission of FIV-B-2542 [9]. FIV-A-Pet infection has been reported to result in lower viral copy numbers and a longer disease-free interval than FIV-B-2542, whereas FIV-C-Pgmr infection has demonstrated high virus burdens and a rapid disease course [15, 16]. Thus, in the present study, we sought to extend, over a broad range of reported phenotypic virulence, the ability of different FIV isolates to cross the placenta and disseminate throughout fetal tissues. Our hypothesis was that FIV-C-Pgmr would be transmitted to fetuses with high frequency and that FIV-A-Pet would be transmitted with low frequency, if at all. Our original aim was to identify FIV isolates that are and are not transmitted to the fetus, to set the stage for future experiments examining the viral determinants of transplacental transmissibility. Unexpectedly, we discovered that both FIV-A-Pet and FIV-C-Pgmr were transmitted to >50% of fetuses, albeit with differences in fetal tissue distribution and proviral burden.

In the present article, we describe in situ assays used to qualitatively examine viral replication in tissues. We report the unexpected finding that fetal FIV infection may occur without
detectable virus in the corresponding placenta. Combined with our prior results that involved FIV-B-2542 [9], we discuss how the FIV model corroborates some assumptions about mother-to-child HIV-1 transmission while challenging others. Most important, we discuss how observations in the FIV system provide clues about mechanisms of perinatal mother-to-child HIV-1 transmission and the types of interventions most likely to be effective in further reducing rates of infant AIDS.

Materials and Methods

Animals and viral inocula. Six female cats (queens) from our specific pathogen-free colony were inoculated intravenously with 100–1000 copies of acute-phase FIV, as described elsewhere [17]. Three queens (3659, 3664, and 3666) were inoculated with FIV-C-Pgmr, and 3 (3866, 3867, and 3395) were inoculated with FIV-A-Pet (a gift from E. Spargur, University of California, Davis). The queens were bred with an FIV-naive tom 4–24 months after infection. Approximately 9 weeks after conception, term fetuses were collected by cesarean section. In each FIV-infected group, 1 queen provided 2 litters, whereas the other 2 queens provided 1 litter each, for a total of 4 litters in each cohort. A total of 11 fetuses from FIV-A-Pet-infected queens and 12 fetuses from FIV-C-Pgmr-infected queens were recovered. As negative control samples, term fetuses and placentas from a sham-inoculated queen were assayed.

Tissue processing and hematologic testing. Placentas and fetuses were surgically harvested, and amniotic fluid and fetal blood were collected as described elsewhere [9]. Specimens analyzed included maternal and fetal blood, placentas, and fetal brain, thymus, liver, spleen, mesenteric lymph node, bone marrow, and blood mononuclear cells. Placentas and fetuses were placed in separate sterile jars with identifying numbers, to match placental tissue with the corresponding fetus in downstream assays. Duplicate tissue samples were collected for polymerase chain reaction (PCR) and histological analyses. Tissues for histologic testing were fixed in 10% neutral buffered formalin, absolute ethanol, or Histochoice-MB (Amresco), washed 5 times with 0.2% Tween (Sigma) in TEN (0.05 M Tris HCl [pH, 7.4], 0.001 M EDTA sodium, and 0.15 M NaCl) on an ELx40 AutoStrip Wash (Bio-Tek Instruments). Wells were blocked with 200 μL of TEN supplemented with 5% vol/vol donkey serum (Sigma) and 2% wt/vol bovine serum albumin (Boehringer-Mannheim) for 48 h at 4°C. After another wash, plates were air dried and sealed with Dynex plate sealer (Dynex Technologies). Plates were stored, long term, desiccated at −20°C. Once thawed, plates were maintained at 4°C.

For detection, 10% of each second-round PCR product was placed in an individual microcentrifuge tube with MHA buffer (2% fetal bovine serum and 0.5% sheared denatured salmon sperm DNA in PBS) quantitation standard (QS) 50 μL. PCR products were denatured in a 95°C heat block for 5 min, then chilled on ice for 5 min. While the PCR products were being denatured and chilled, biotinylated probe was bound to the avidin-coated plates. The probe, CB gag503 (biotin-5′-TCACCTCTAAACCTTCTCTTGC-3′), is reverse and complementary to bases 481–503 of the FIV gag gene sense strand. Fifty microliters of well of probe, diluted to a final concentration of 50 pg/mL in MHA buffer, was incubated at 37°C for ~10 min. After the PCR products had chilled, excess probe was washed from the plates 5 times with 0.2% Tween/TEN. The PCR product solutions were added to the wells and hybridized at 37°C for 30 min. Plates were washed, and 50 μL/well of 1:1000 horseradish peroxidase-conjugated anti-digoxigenin fragment antibody–binding (Roche Molecular Biochemicals) was bound for 20 min at 37°C. After washing, peroxidase was detected with 3,3′,5,5′-tetramethylbenzidine/H2O2 (Kirkegaard & Perry) for 2–5 min. Reactions were stopped with 2.5 M H2SO4. Optical densities (ODs), measured by absorbance at 450 nm (reference, absorbance at 570 nm), were recorded using a Dynatech 5000MR microplate reader (Dynatech Technologies). Positive reactions were defined as those with a minimum OD of 0.1. The optical reader was blanked on the naive DNA negative control well, which was <0.02 OD or the assay was repeated. By testing dilutional series of known FIV gag plasmid copies numbers, we determined that our PCR protocol approached a sensitivity of 1 provirus copy/μg DNA (Rogers, A. B., unpublished data). Therefore, semiquantitative DNA PCR results (provirus copies/μg DNA) were recorded in factors of 10 on the basis of the highest log dilution that yielded a positive result (1 copy, 10 copies, 100 copies, etc.). Our case definition for fetal FIV infection was a positive DNA PCR result on any tissue, excluding placenta.

Generation of RNA competitor by substitutional PCR. Amniotic fluid and maternal and fetal plasma samples were tested for viral RNA load with a quantitative competitive reverse-transcription (RT)–PCR (qRT-PCR) assay. Because of the limited blood volume that could be collected from the feline fetuses, we developed a single-sample method to quantitate viral RNA in plasma. We used substitutional PCR to generate the competitor RNA molecule. The competitor was developed by substituting a foreign 23-base sequence in the wild-type second-round sense gag PCR product. The substituted foreign sequence (FeLV 1776; 5′-TGACCAAAGCTTTCCCTTGACC-3′) was derived from bases 1754–1776 of the feline leukemia virus (FeLV) gag gene. Software-assisted (MacVector; Oxford Molecular Group) analysis of the FeLV sequence demonstrated no significant homology with FIV or endogenous cat genome sequences in the GenBank database. The substitutional PCR process involved a series of internal amplifications of the
second-round gag product beginning at base 507 and working 5′ back to the gag3 primer origin (base 446), trailing 5′ sequences from the ends of primers in successive steps (figure 1).

We first amplified a subunit of the second-round gag PCR product by substituting primer gag507 (5′-AGTTCAATTAGTTTACAGCCTTT-3′) for gag3. The next round of PCR used primer gag507 with a trailing 5′ foreign (FeLV 1776) 23-base sequence (5′-CCCAGTCCCTTTTTCGAAGCAGTGACCCCTTTTCGAAGCAGTAGTTCAATTATGGTTTA-3′). To by trailing the FeLV 1776 sequence from the gag507 primer, we incorporated the foreign sequence into all amplified products. We then “added back” the original gag3 primer binding sequence by trailing it from the 5′ end of FeLV 1776 (5′-TTGACCCAATAATGTTGTCACCAGTTCCTTTTTCGAAGCAGTAGTTCAATTATGGTTTA-3′). In the last step, we trailed a T7 RNA polymerase promoter sequence with a 5′ GCG cap and 3′ 10-base linker (5′-GGCGGATATCAGCCTCTTCTCCAGTTTACAGCAGTTTTGCTTTCGGAAGCAGTAGTTCAATTATGGTTTA-3′) to the 5′ end of gag3 (figure 1). We performed direct in vitro transcription of the final PCR product with the Ampliscribe T7 High Yield Transcription Kit (Epicentre). Residual DNA was digested with RQ1 RNase-free DNase (Promega). RNA was purified with the Qiamp Viral RNA Mini Kit (Qiagen) and quantitated on a DU-70 spectrophotometer (Beckman Instruments). RNase-free 1X Tris-EDTA buffer (pH, 7.0; Sigma) was added to the purified RNA for a final concentration of 10^5 molecules/μL. Individual aliquots were frozen at −70°C for long-term storage. Aliquots were thawed and diluted to 100 copies/μL in RNase-free water immediately prior to qRT-PCR.

Viral RNA quantification. To determine the viral RNA load in fluids, RNA was purified from 200 μL amniotic fluid or EDTA-plasma samples using the Qiamp Viral RNA Mini Kit (Qiagen) and quantitated on a DU-70 spectrophotometer (Beckman Instruments). qRT-PCR was performed in a P-E 9600 thermocycler (PE Applied Biosystems) using the primers digoxigenin-gag3 and -gag4 (Sigma Genosys). After a 30-min RT step at 65°C, the samples were cycled 40 times at 94°C for 10 s, 60°C for 30 s, and 50°C for 30 s, with a final 72°C extension for 4 min.

PCR products were quantitated in 96-well microplate hybridization assay plates, as described above, with the following modifications: CB gag503 probe (biotin-5′-TCACCTCCTAACCCTTCTCTGC-3′) was placed in the first 5 wells of a 96-well column. FeLV 1776 probe (biotin-5′-ACTGCTTCGAAAAGGGAACTGGG-3′), which is complementary to the substituted FeLV sequence in the RNA competitor molecule, was placed in lanes 6 and 7. A mixture of wild-type and competitor probe was placed in lane 8, which served as the negative control. Ten percent of each PCR product was added to each PCR solution. PCR solutions were derived from the GeneAmp EZrTth RNA PCR Kit (PE Applied Biosystems). RT-PCR was performed in a P-E 9600 thermocycler (PE Applied Biosystems) using the primers digoxigenin-gag3 and -gag4 (Sigma Genosys). After a 30-min RT step at 65°C, the samples were cycled 40 times at 94°C for 10 s, 60°C for 30 s, and 72°C for 30 s, with a final 72°C extension for 4 min.

To calculate viral RNA copies/mL, we first calculated a QS average, using the following formula: QS = [OD lane 6 + 5 × OD lane 7]/2. To determine the number of wild-type RNA copies, we took the highest dilution lane of wild-type PCR product (lanes 1–5) with an OD >0.1, multiplied the OD by the dilution factor (e.g., 5×, 25×, 125×, etc.), and divided that figure by the quantitation standard average. The final figure was multiplied by 1000 (100 copies competitor/0.1 mL input plasma RNA) to determine viral RNA viral load in copies/mL. Samples below the lower detection threshold of 100 copies/mL were assigned a value of 40 copies/mL for statistical analysis.

Fluorescence immunohistochemistry. Our fluorescence immunohistochemistry protocol is described in detail elsewhere [19]. In brief, ethanol- and Histochoice-fixed tissue sections were deparaffinized and rehydrated. Endogenous peroxidase was quenched with 3% H2O2, and sections were blocked with 1% each naive goat and cat serum in TNB blocking buffer (TSA System; NEN) and anti-FIV antibodies in high-titer plasma from a cat chronically infected with FIV-A-Pet and 1 mg/mL biotin-conjugated protein A (Sigma). While the tissue sections were being blocked, anti-FIV antibodies in high-titer plasma from a cat chronically infected with FIV-A-Pet and 1 mg/mL biotin-conjugated protein A (Sigma) were bound in solution at room temperature. Immediately prior to placement on tissues, a final dilution to 1:1000–1:10,000 was done. Primary antibody was incubated on tissues for 2 h. Signal was tyramide-amplified with Cy3-TSA (NEN). Nuclei were counterstained with 1 μg/mL 4′,6-diamidino-2-phenylindole hydrochloride (Sigma). Slides were...
Figure 2. Comparison of hematologic parameters in blood from feline immunodeficiency virus (FIV)-clade A Petaluma (FIV-A-Pet)– or clade C FIV PaddyGammer (FIV-C-Pgmr)–infected queens at the time of cesarean delivery. PBMC, peripheral blood mononuclear cells; WBC, white blood cells. *, Statistically significant difference between FIV-A-Pet and FIV-C-Pgmr cohorts.

partially air dried and mounted with Vectashield (Vector Labs). As negative control samples, FIV antibody–positive serum was applied to FIV-naive tissues, and FIV antibody–negative serum was applied to FIV-positive tissues. Positive control tissues were derived from a group of juvenile cats in the acute-phase of FIV-C-Pgmr infection that had high plasma and tissue viral titers [19].

RNA and DNA in situ hybridization. Viral RNA was demonstrated in formalin-fixed tissue sections using a FIV RNA in situ hybridization protocol described elsewhere [20]. DNA in situ hybridization was done by modifying the RNA in situ hybridization protocol to include a 90°C heat denaturation step for 30 s–3 min immediately after probe application. Sense probe was used for DNA in situ hybridization. In our hands, ethanol-fixed tissue sections without protease digestion proved most sensitive for DNA in situ hybridization. FIV-naive negative control tissues were included in all assays. For RNA in situ hybridization, the signal was detected with Vector VIP, and slides were counterstained with methyl green (Vector Labs). For DNA in situ hybridization, the signal was detected with Vector NovaRed and slides were counterstained with Gill’s hematoxylin (Vector Labs).

Assessment of FIV replication in cultured placental cells. Surgically harvested FIV-infected and naive placentas were homogenized, and cell suspensions were cultured as described elsewhere for virus isolation [21], except that naive blood mononuclear cells were omitted from the cultures. Placental cell samples were collected at 0, 3, and 7 days, spun down onto glass slides, fixed with acetone, and assessed for FIV antigens by immunocytochemistry using murine anti-FIV capsid (gag) monoclonal antibody vpg 50 (Serotec) and peroxidase-conjugated anti-mouse IgG (Sigma). Prior to antibody binding, endogenous peroxidases were inactivated with 3% H2O2/PBS. Signal was detected with Vector VIP and cells were counterstained with methyl green (Vector Labs). FIV in supernatant was detected by ELISA, as described elsewhere [9].

Results

Maternal hematology and virus loads. Mean maternal hematocrit and serum protein values at the time of cesarean delivery were virtually identical between the FIV-A-Pet and FIV-C-Pgmr cohorts (figure 2). The FIV-A-Pet group had a higher mean CD4+ T cell count and a lower CD8+ count, although these values individually were not statistically significant. However, there was a highly significant difference in CD4+/CD8+ T cell ratios between the groups. The mean ratio for the FIV-A-Pet queens was 1.3, whereas the mean for the FIV-C-Pgmr group was 0.6 (P = .003; figure 2). We found higher plasma viral RNA loads in the FIV-C-Pgmr cats than the FIV-A-Pet cohort, although this difference was not statistically significant.
The FIV-C-Pgmr queen mean blood mononuclear cell provirus load, by contrast, was significantly higher than that in the FIV-A-Pet group ($P = .01$; figure 2).

**Fetal and placental FIV.** Eight (67%) of 12 fetuses from the FIV-A-Pet cohort and 10 (91%) of 11 fetuses from the FIV-C-Pgmr cohort were DNA PCR–positive (figure 3). We detected no viral RNA by RT-PCR in fetal plasma or amniotic fluid from either group (data not shown). Unlike the FIV-C-Pgmr group, we never detected virus in brain or liver from FIV-A-Pet fetuses. Viral DNA prevalence, tissue distribution, and DNA loads were higher in the FIV-C-Pgmr group than in FIV-A-Pet fetuses (figure 3). All placentas from the FIV-C-Pgmr group were DNA PCR–positive and carried a mean proviral burden equivalent to that of purified maternal blood mononuclear cells. By contrast, fewer than half of placentas from the FIV-A-Pet group were DNA PCR–positive, with a mean placental proviral burden $\sim 1$ log lower than that in maternal blood mononuclear cells. Surprisingly, 5 of (63%) 8 FIV-A-Pet DNA PCR–positive fetuses were associated with a DNA PCR–negative placenta (figure 3). A summary of the prevalence and distribution of FIV DNA in fetuses is demonstrated in figure 4; results from a study elsewhere with FIV-B-2542 [9] are included to demonstrate the differences in term fetal FIV DNA prevalence and tissue distribution among these 3 virus isolates.

**DNA in situ hybridization.** We identified FIV-C-Pgmr DNA in fetal-derived (chorioallantoic) placentas by DNA in situ hybridization (figure 5A). In contrast, we rarely detected viral DNA in placental sections from the FIV-A-Pet group. The rare viral DNA–positive cells we did encounter in FIV-A-Pet placentas were usually intravascular, and, because maternal vessels are retained in the feline placentas, these cells may have been circulating leukocytes from the mother (figure 5B). Within FIV-C-Pgmr–positive fetuses, we detected viral DNA–positive cells by in situ hybridization in multiple organs, including thymus (figure 5C) and brain (figure 5D). Intracerebral FIV-C-Pgmr DNA was usually located in cells with glial morphology in cortical gray matter or in the hippocampus and nearby periventricular sites (figure 5D). In the FIV-A-Pet–positive fetuses, we only visualized viral DNA by in situ hybridization in the thymus, although other organs (excluding brain and liver) were fluid-phase DNA PCR–positive. In both the FIV-C-Pgmr and FIV-A-Pet groups, most viral DNA–positive thymocytes were fluid-phase DNA PCR–positive. In both the FIV-C-Pgmr and FIV-A-Pet groups, most viral DNA–positive thymocytes were located in the thymic cortex (figure 5C). Thymic medullary FIV DNA was usually localized to cells within or near Hassal’s corpuscles. Our assay did not distinguish between integrated and unintegrated FIV DNA, although the signal was always localized in cell nuclei.

**RNA in situ hybridization and immunohistochemistry.** We
did not detect viral transcripts by RNA in situ hybridization in placental (figure 6A) or fetal (figure 6B) tissues. Likewise, we identified no cell-associated FIV antigens by fluorescence immunohistochemistry in placental (figure 6D) or fetal (figure 6E) tissue sections. Control tissues from acutely infected cats assayed in parallel were appropriately positive by both methods (figure 6C and 6F).

Placental cell ex vivo culture. From primary FIV-naive placental cell cultures, virus was not detected at any time point (not shown). Placental cells from FIV-infected queens contained no detectable viral antigens on day 0 (figure 7A). However, using immunocytochemistry, we detected FIV antigens within 3 days after inoculation. After 1 week of in vitro culture, many placental cells contained abundant intracytoplasmic FIV capsid (gag-encoded) proteins (figure 7B). Productive cellular infection was confirmed by the detection of free virus in day 7 supernatants by antigen ELISA (data not shown).

Discussion

We report here on maternal-to-fetal transmission of FIV isolates representing clades A and C. Our results suggest that vertical FIV transmission is not clade-dependent, is often occult, and may occur more frequently than has been suggested by prior FIV studies that focused only on postnatal detection [22–24]. Among virus isolates we have studied in our laboratory, FIV-C-Pgmr has produced the highest fetal tissue provirus burdens, followed by FIV-B-2542 [9] and, finally, FIV-A-Pet (figure 4). Uniquely, FIV-A-Pet was never demonstrated in fetal brain or liver and was infrequently detected in placenta, which suggests a cellular tropism different than the clade B and C isolates.

Placental localization of FIV appeared to be neither necessary nor sufficient for fetal infection. FIV-C-Pgmr DNA was detected in all placenta and in 92% of fetuses. In a study elsewhere, we detected FIV-B-2542 in 96% of placenata but in only 60% of fetuses [9]. In contrast, our DNA PCR assay detected FIV-A-Pet in only 42% of placenta but in 67% of fetuses, and there was no concordance between the presence of placental and fetal virus. By in situ hybridization, we demonstrated FIV-C-Pgmr DNA in cells of the fetal-derived placental chorioallantois. We were not surprised by that result, because the levels of FIV-C-Pgmr provirus detected by fluid-phase DNA PCR were too high to be accounted for solely by circulating maternal leukocytes. In contrast, we rarely detected infected cells by DNA in situ hybridization in FIV-A-Pet placentas, even though two-thirds of fetuses were PCR-positive, and those placental cells that did contain viral DNA may have been maternal leukocytes.

We detected neither FIV antigens nor transcripts in fetal and placental tissue sections by immunohistochemistry and RNA in situ hybridization. This suggests that, in these tissues, provirus was latently integrated or that viral replication was below our level of detection. We observed up-regulated viral replication after ex vivo activation of placental cells by immunocytochemistry and ELISA, although, like PCR, this assay could not distinguish between fetal and maternal virus. Although functionally competent provirus was present in the placenta, there was little evidence of active in vivo replication. In a study elsewhere, we demonstrated by coculture that the viral DNA detected by PCR analysis in both fetal and placental tissue sources represented competent virus [9].

We have shown here and elsewhere [9] that term fetuses frequently exhibit an occult infection pattern, defined as the sequestration of FIV in tissues in the absence of detectable virus in plasma or circulating blood cells. Depending on the isolate in question, 27%–83% of fetuses with demonstrable tissue-associated viral DNA do not have detectable FIV in circulating leukocytes, and no fetuses we have tested have demonstrated detectable plasma viral RNA. Thus, blood tests alone are inadequate to document the presence of FIV in term fetuses. This is significant, because prevailing definitions on the timing of vertical HIV-1 transmission rely heavily on serial neonatal and infant blood assays [25, 26]. If fetal HIV-1 tissue sequestration parallels that of FIV, current definitions underestimate in utero HIV-1 infections and overemphasize intrapartum virus trans-
mission, as has been suggested in a recent meta-analysis of perinatal HIV chemoprophylaxis trials [27]. Particularly worrisome from a reservoir standpoint is the known tropism of some HIV-1, SIV, and FIV isolates for cells in the fetal brain [9, 28–30]. Until more is known about viral tissue sequestration in human fetuses, conclusions are best considered speculative regarding the timing of perinatal HIV-1 transmission on the basis of blood assays alone.

If perinatal HIV-1 transmission, like FIV, commonly occurs in utero, intervention strategies directed solely against intrapartum virus exposure should little reduce infant infections. There is evidence that this is the case. Peripartum vaginal chemical disinfection has routinely disappointed as a strategy for reducing vertical HIV-1 transmission [31]. Reports on the benefit of cesarean delivery are mixed. Although most studies have shown some reduction in HIV prevalence in cesarean-delivered versus vaginally delivered infants [32–34], other studies have demonstrated little benefit from this invasive procedure [35]. For maximal effectiveness, it appears that cesarean delivery should be performed prior to the onset of labor contractions. It has been suggested that the chief benefit of early cesarean delivery may be the prevention of late intrauterine, rather than intrapartum, HIV-1 transmission [27]. Currently, there is no universally accepted estimate of intrapartum HIV-1 transmission on the basis of reductions attributable to cesarean delivery.

If most vertical transmission of HIV-1 occurs in utero, why are many infants protected from disease even when chemoprophylaxis is not begun until at or near the time of birth [36, 37]? One answer could lie in the viral replication kinetics in the fetus. We identified FIV DNA, but not viral antigens or RNA, in fetal tissues. Likewise, antigens in placental cells were not produced in detectable amounts unless the cells were activated ex vivo. Basal replication rates of many HIV-1 isolates in human fetuses may be likewise low. In fact, the observation that human infants with detectable HIV at birth progress to AIDS and death faster than children whose first positive test occurs later in infancy [38] may simply be a reflection of the replicative ability of a given virus in the fetus rather than being related the timing of vertical transmission. Moreover, there is evidence that human fetuses may respond to intrauterine HIV-1 exposure through innate and/or acquired immune mechanisms [39, 40], including the finding of persistently HIV-negative infants born with HIV-specific cyto-
Figure 6. Feline immunodeficiency virus (FIV) RNA in situ hybridization (A–C) and fluorescence immunohistochemistry (D–F). A, B, D, and E, Placental and fetal tissues from clade C FIVpaddyGammer (FIV-C-Pgm) DNA-positive specimen. Positive control tissues are thymus (C) and bone marrow (F) from a juvenile cat acutely infected with FIV-C-Pgm. A, No detection of FIV RNA transcripts in placenta. B, No FIV RNA in fetal thymus. C, Scattered FIV RNA-positive cells (purple) in the positive control tissue. D, No detection of FIV antigens in placental cells. E, No detection of FIV antigens in fetal thymus. F, Abundance of FIV antigen–positive cells in bone marrow from a positive control cat. Fluorochrome key: cell nuclei, 4′,6′-diamidino-2-phenylindole hydrochloride (blue); and FIV, Cy3 (red). Bar, 100 μm (A and D); 200 μm (B and E); or 50 μm (C and F).
toxic T lymphocyte responses [41, 42]. Thus, a low basal viral replication rate coupled with host antiviral responses could serve to contain the spread of HIV-1 in most fetuses. Initiation of chemotherapy at the time of birth, when viral up-regulation would be expected to coincide with immune activation, might be sufficient for maintaining viral suppression during the early postnatal period. A similar scenario has been proposed in the case of peripartum HIV-1 transmission [27]. Postexposure prophylaxis has demonstrated promising efficacy in protecting neonatal macaques infected with SIV [43–47]. In short, our results and those of others suggest that antiretroviral chemotherapy should remain the mainstay of programs aimed at reducing infant HIV-1 infections.

In conclusion, we have demonstrated intrauterine transmission of multiple FIV isolates, albeit with variability in transmission prevalence, tissue distribution, and fetal proviral loads. Virus isolates tropic for placenta demonstrated the broadest fetal dissemination and highest fetal tissue levels. However, FIV localization to placenta was neither necessary nor sufficient for fetal infection. Occult infection, defined as FIV sequestration in tissues without detectable circulating virus, was common. As such, blood assays unreliably detected intrauterine infection. Taken together, our findings join those of others in raising doubts about defining the timing of vertical HIV-1 transmission on the basis of serial postnatal blood assays [27]. If, like FIV, most perinatal HIV-1 transmission occurs in utero, intervention should focus on containing viral replication in the fetus and neonate. Further work is required to determine the proportion of fetuses exposed to HIV or FIV who do not develop established pediatric infections. Future studies in the FIV system may help identify the host and viral factors that together determine whether the final outcome of fetal lentivirus exposure is progressive infection, regressive infection, or complete virus clearance and may help identify new targets for the intervention of mother-to-child HIV-1 transmission.

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References