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Feline immunodeficiency virus infection of cats as a model to test the effect of certain in vitro selection pressures on the infectivity and virulence of resultant lentivirus variants

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Summary

Three groups of specific pathogen-free (SPF) domestic cats, each containing 5 animals, were infected with one of three closely related FIV variants and monitored for 36 weeks. A fourth group of 5 cats was sham-infected and served as uninfected controls. FIV variants included: (1) a fully virulent animal passaged FIV-Petaluma; (2) a Crandell feline kidney (CrFK) cell-adapted FIV-Petaluma (FIV-CrFK); and (3) a variant of FIV-CrFK (FIV-CrFK$_{AZT}$) that had been selected in vitro for resistance to azidothymidine. Cats infected with fully virulent FIV-Petaluma strongly seroconverted, became persistently viremic, and exhibited lymphadenopathy, neutropenia, and inversion of the CD4+:CD8+ T cell ratio. Cats infected with FIV-CrFK seroconverted but the antibody responses were much weaker and more variable; two of the cats became transiently viremic and no hematologic abnormalities or clinical signs of illness other than a very mild lymphadenopathy were observed. None of the five cats inoculated with FIV-CrFK$_{AZT}$ seroconverted, became viremic, or exhibited any gross or hematologic signs of disease, even though proviral DNA was transiently detected in tissue following inoculation. This study demonstrates that the FIV infection model can be used to assess differences in the virulence of FIV variants, including variants selected for antiretroviral drug resistance.

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Feline immunodeficiency virus; FIV; Acquired immunodeficiency syndrome; AIDS; Cat diseases; Azidothymidine; AZT; T-lymphocytes; T4 lymphocytes; Drug resistance

Introduction

Antiviral drugs such as 3′-azido-3′deoxythymidine (AZT, zidovudine) have shown efficacy in human immunodeficiency virus (HIV) infection (Fischl et al., 1987; Japour and Crumpacker, 1991; Richman, 1991). However, this beneficial effect is often curtailed by the emergence of AZT-resistant variants of HIV (Boucher et al., 1990; Land et al., 1990; Larder et al., 1989; Richman et al., 1990; Rooke et al., 1989). Resistance to other drugs such as dideoxyinosine (ddI) has also been recently described (St. Clair et al., 1991). Antiviral drug resistance results from the cumulative acquisition of codon mutations within the reverse transcriptase (RT) gene of HIV (Larder and Kemp, 1989; Larder et al., 1991). Selection pressure from anti-retroviral drugs is likely to add to the constant selection pressure of the host's immune system, which favors the emergence of progressively more virulent strains within the same host (Anderson, 1991; Nowak et al., 1991).

The clinical implication of drug resistance depends ultimately on the biological behavior of the resistant HIV variants. If drug resistant mutants are less virulent, continued drug therapy would be indicated. However, if drug resistant variants are equally or more virulent than parental strains, drug therapy should be suspended. Clinical observations have not proven helpful in determining the biological implications of antiviral drug resistance. Although abrupt deterioration has not been observed following the emergence of drug-resistance in adults, there is a negative correlation between AZT sensitivity and clinical outcome among HIV-infected children (Bach, 1990; Tudor-Williams et al., 1992). Unfortunately there is no easy way to determine the virulence of drug resistant HIV variants directly in humans or in animals. Therefore, an animal model using a closely related retrovirus would be of particular value.

The RT of feline immunodeficiency virius (FIV), a lentivirus, is identical to the RT of HIV in physical and catalytic properties and in sensitivity to various RT inhibitors (North et al., 1989; 1990a,b; Remington et al., 1991; Cronn et al., 1992). The disease induced by FIV in domestic cats is also similar to that caused by HIV in people (reviewed by Pedersen, 1993). There is a mild primary illness akin to the initial mononucleosis-like illness of HIV infection (Yamamoto et al., 1988), which is followed by an asymptomatic period lasting for years. This asymptomatic period is associated with a progressive decline in CD4+ T cell numbers and inversion of the CD4+:CD8+ T cell ratio, diminished lymphocyte responsiveness to IL-2 and phytomitogens, decreased antibody responses to T-dependent immunogens, hypergammaglobulinemia and diminished IL-2 production (Ackley et al., 1990; Barlough et al.,

1991; Novotney et al., 1990; Siebelink et al., 1990; Torten et al., 1991). Drug resistant mutants of FIV have also been induced in vitro (Remington et al., 1991). One drug-resistant mutant, FIV-CrFK$_{AZT}$, mimics the AZT-resistant clinical isolates of HIV-1 in 3′-azidonucleosides and other antiviral nucleosides (Remington et al., 1991).

The aim of the present study was to demonstrate the utility of the FIV model for comparing differences in the infectivity and virulence of several wild-type, tissue culture adapted and antiviral drug selected strains. The design of the study precluded any determinations on the relationship of the drug-resistant phenotype to virulence. However, the study demonstrated several important pitfalls in animal experiments of this type and showed the ease and sensitivity of the model in comparing variant strains of FIV to each other.

Materials and Methods

Virus stocks

Virulent, non-cell culture adapted FIV-Petaluma was used in the form of infected whole heparinized blood from SPF cat no. 39 (Pedersen et al., 1987; Yamamoto et al., 1988). FIV-Petaluma was adapted for in vitro replication on Crandell feline kidney (CrFK) cells and designated FIV-CrFK. FIV-CrFK replicates well in feline macrophages in vitro but has lost considerable infectivity for feline T lymphocytes (data not shown).

An AZT-resistant variant of FIV-CrFK, designated FIV-CrFK$_{AZT}$, was selected by propagating FIV-CrFK in the presence of 10 uM AZT (Remington et al., 1991). Culture propagation of the CrFK adapted FIV-Petaluma was done in parallel, both in the presence and absence of AZT. Therefore, FIV-CrFK and FIV-CrFK$_{AZT}$ stocks used in this study were from identical passage levels in CrFK cells. The FIV-CrFK$_{AZT}$ variant was fully infectious in vitro as judged from its ability to induce infectious foci and reverse transcriptase (RT) activity upon cell-free passage to non-infected cultures (Remington et al., 1991). FIV-CrFK$_{AZT}$ was cross-resistant to 3′-azido-2′,3′-dideoxyuridine (AZdU) and 3′-azido-2′,3′-dideoxyguanosine (AZG) but was sensitive to several other compounds, including 2′,3′-dideoxyinosine (ddI) and phosphonoformate (PFA), suggesting that resistance was linked to the presence of the 3′-azido group on the nucleoside (Remington et al., 1991). This pattern of resistance and cross-resistance was similar to that for most AZT-resistant clinical isolates of HIV that have been described (Larder and Kemp, 1989; Larder et al., 1990).

Animals and animal inoculations

Twenty SPF domestic cats, from 6–8 months of age, were obtained from the breeding colony of the Feline Retrovirus Research Laboratory, School of Veterinary Medicine, University of California-Davis, and were housed in small groups in pathogen-free isolation facilities provided by the Animal Resources

Service at the same institution. The animals were neutered 2 weeks prior to being put on study and were randomized by age, sex, and litter of origin into four groups of five cats each.

Cats were inoculated by the intraperitoneal (IP) route according tc the following scheme:

Group 1: 3 ml of uninfected CrFK cell supernatant (sham-infected controls).
Group 2: 2×10^6 RT units (cpm) of FIV-CrFK$_{AZT}$ in 3 ml medium.
Group 3: 2×10^6 RT units (cpm) of FIV-CrFK in 3 ml medium.
Group 4: 0.5 ml of blood from SPF cat no. 39, chronically infected with FIV-Petaluma. Cats in groups 2 and 3 were reinjected with 10^6 RT units of their respective inocula 24 weeks following the initial inoculations.

Virus isolation using whole-blood pre-culture

Feline blood samples (approximately 1 ml) were collected by jugular venipuncture into sterile glass tubes containing preservative-free sodium heparin. The blood was centrifuged ($400 \times g$) for 5 min and the plasma was withdrawn and stored at $-20°C$. For each sample, the blood cells were added to 9.5 ml of growth medium containing 1:1 Leibovitz's L-15 medium and Dulbecco's modified Eagle medium, 10% bovine fetal serum (HyClone Laboratories, Logan, UT), 2 mM L-glutamine, 1% non-essential amino acids, 200 U/ml recombinant human interleukin-2 (kindly provided by Cetus Corporation, Emeryville, CA), 1 μg/ml concanavalin A (Con A), 100 IU/ml penicillin, and 10 μg/ml streptomycin. The cultures were placed into sealed 25-cm^2 flasks and incubated at 37°C for 3–5 days. Cells were then layered onto Ficoll gradients (10 ml culture:3 ml Ficoll) and centrifuged at $1000 \times g$ for 20 min. Peripheral blood mononuclear cells (PBMC) isolated from the interface were resuspended in 5 ml of RPMI medium, pelleted by centrifugation, and washed twice. The washed pellets were resuspended in 1 ml of growth medium. The cells were transferred in duplicate to 24-well plates and co-cultivated with freshly passaged, one-half confluent CrFK cells. Cultures were sealed in plastic bags and incubated at 37°C in 5% CO_2. They were fed fresh medium every 2–3 days and sampled once weekly for 6 weeks. After the first week the Con A was removed from the medium. The weekly samples of cell culture supernatant were tested for FIV core antigen (p24) by an antigen-capture ELISA, as described previously (Dandekar et al., 1992).

Polymerase chain reaction for the detection of FIV proviral DNA

Lysates of bone marrow and/or lymph node were analyzed for FIV proviral DNA at different time points following inoculation using a double or nested polymerase chain reaction (PCR) (Saiki et al., 1988). Bone marrow was collected from the neck and shaft of the femur with a bone marrow needle and placed immediately into heparinized tubes. A popliteal lymph node was removed at the termination of the study. Bone marrow (0.1 ml) and lymph node tissue (50 mg) were lysed for 3 h at 56°C in lysis buffer [10 mM Tris-HCl (pH 8.3), 0.45% NP 40, 0.45% Tween-20, proteinase K (50 μg/ml)], and the

DNA was phenol-chloroform extracted.

Two pairs of primers, external and internal, were produced to the *gag* region of the FIV genome based on available sequences (Talbott et al., 1989). The external primers (first round) were from nucleotide positions 929–948 (5′-CTACTGCTGCTGCAGCTGAA-3′) and 1375–1394 (3′-CGTGGTCGATCCTACGTCAC-5′), while internal primers (second round) were from nucleotide positions 1236–1255 (5′-GGATGAAAGCTTAAAG-CAAC-3′) and 1325–1394 (3′-CACTGCATCCTAGCTGGTGC-5′). For the first round of amplification, 150–500 ng DNA from bone marrow or lymph node were added to 19.25 μl of PCR reaction mixture consisting of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, 200 mM of each dNTP, 15 pmol of each primer and 1.25 units of *Taq* polymerase (Perkin Elmer Cetus, USA). The PCR reactions were adjusted with deionized water to a final volume of 50 μl. The DNA was denatured prior to amplification by incubation for 5 min at 94°C. Samples were amplified for 35 cycles in PCR (1 min denaturation at 94°C, 1 min annealing at 55°C and 1.5 min primer extension at 72°C). Two μl of the resulting PCR products were used under identical reaction conditions as template for the second round of amplification (35 cycles). DNA from known FIV positive and negative cats were used as controls in each PCR reaction. Ten μl of each PCR product were analyzed by electrophoresis through 2% agarose/0.5× Tris-borate/EDTA electrophoresis buffer (TBE) followed by ethidium bromide staining.

Serology and hematology

Serum antibodies to FIV were detected using an indirect immunofluorescence procedure with FIV-infected CrFK cells, as described previously (Yamamoto et al., 1988). Sera were tested at a dilution of 1:10 and antibody levels reported as negative (−), weakly positive (+/−), or strongly positive (+). Certain positive samples were also tested by Western blot.

Total leukocyte counts in feline blood samples were determined by impedance using a System 9000 cell counter (Baker Instruments, Allentown, PA, USA). Lymphocyte and neutrophil counts were subsequently obtained from Wright-Leishman-stained blood smears using standard enumerative methods (Barlough et al., 1991).

Enumeration of CD4+ and CD8+ lymphocyte subsets

Murine monoclonal antibodies (Mabs) to feline CD4 and CD8 T-cell surface markers were used for enumeration of T-lymphocyte subsets, as described (Ackley et al., 1990; Barlough et al., 1991). The CD4+:CD8+ cell ratios were enumerated by flow cytometric analysis. Mean values were calculated for use in statistical analysis (Student's *t*-test, Mann-Whitney *U*-test).

Results

Signs of FIV-induced disease

Clinical signs of illness were not observed in the sham- or FIV-CrFK$_{AZT}$-inoculated cats (groups 1 and 2). Three of five cats inoculated with FIV-CrFK (group 3) developed a mild, transient, generalized lymphadenopathy within 12 weeks of inoculation. All five cats inoculated with non-cell culture-adapted FIV-Petaluma (group 4) developed mild to moderately intense, generalized lymphadenopathy that persisted for several months.

Total lymphocyte counts did not differ significantly among the four groups (Fig. 1). Cats in group 4, however, exhibited a decrease in total neutrophil numbers, which reached a nadir at week 8 and remained significantly depressed below control levels for most of the study (Fig. 2).

Lymphocyte subsets were examined in sequential blood samples collected over the period of the study (Fig. 3). These data revealed a statistically significant decrease in the CD4+:CD8+ T-cell ratio in cats infected with FIV-Petaluma (group 4), beginning within 8 weeks of infection. As expected, this abnormality was due to an absolute decrease in CD4+ T cells and a normal to increased number of CD8+ T cells (data not shown). The CD4+:CD8+ T-cell ratios in cats infected with FIV originating in cell culture (groups 2 and 3) showed no significant alterations when compared to sham-infected controls.

Virus isolation

Virus was not recovered from the PBMC of cats in group 1 (controls) or group 2 (FIV-CrFK$_{AZT}$) (Table 1). Even after a second inoculation with the

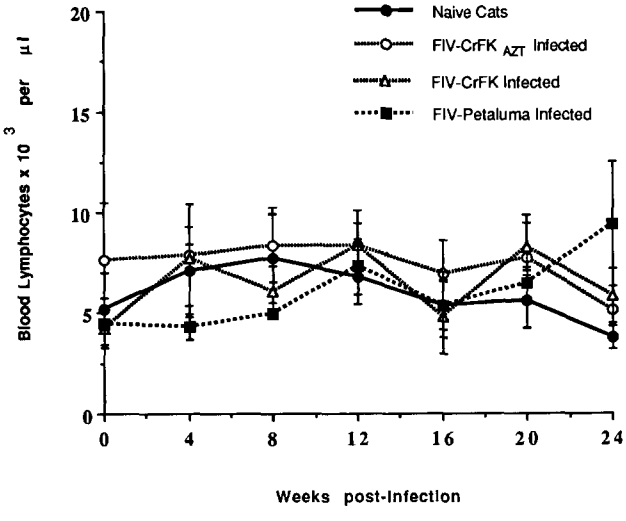

Fig. 1. Serial lymphocyte counts in control and inoculated SPF cats. Each data point represents the mean counts for 5 cats ± 1 S.E.M.

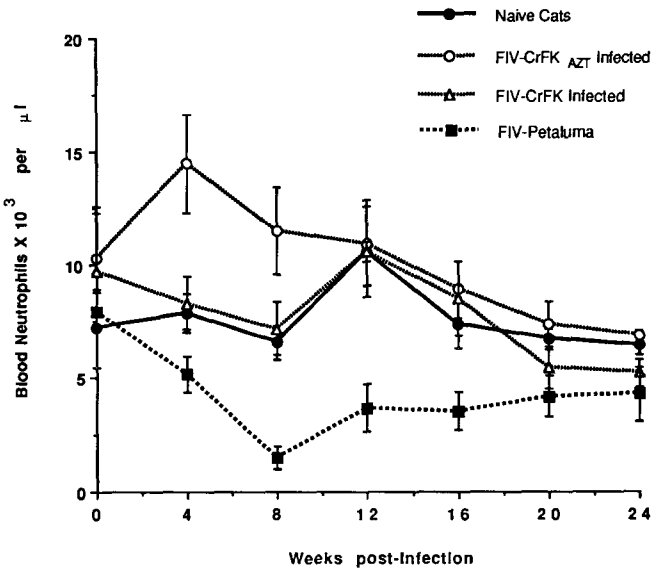

Fig. 2. Serial neutrophil counts in control and inoculated SPF cats. Each data point represents the mean counts for 5 cats ± 1 S.E.M.

AZT-resistant variant at week 24, cats in group 2 remained virus isolation-negative. In group 3 (FIV-CrFK), three cats had demonstrable cell-associated

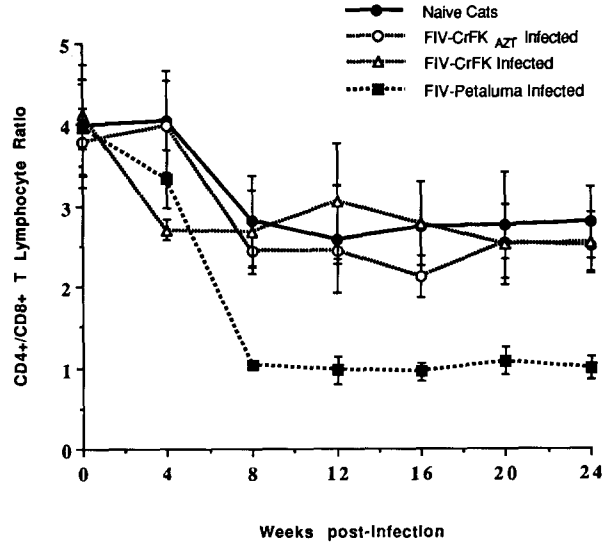

Fig. 3. Serial CD4+:CD8+ T-cell ratios in control and inoculated SPF cats. Each data point represents the mean ratios for 5 cats ± 1 S.E.M.

TABLE 1

Isolation of FIV from peripheral blood mononuclear cells of experimentally inoculated SPF cats

cats	Weeks post-inoculation													
	0	2	4	6	8	10	12	16	20	26	28	30	32	36
Group 1 (sham-infected controls)														
All cats	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Group 2 ($FIV\text{-}CrFK_{AZT}$)														
All cats	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Group 3 (FIV-CrFK)														
5003	−	−	−	−	−	−	−	−	−	−	−	−	−	+
5013	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5017	−	−	+	−	−	−	+	−	−	−	−	−	−	+
5029	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5032	−	−	−	−	−	−	+	+	−	+	+	+	+	+
Group 4 (FIV-Petaluma)														
5000	−	+	+	+	+	+	+	+	−	+	−	+	+	−
5010	−	+	+	+	+	+	+	+	+	+	+	+	+	+
5016	−	−	+	+	+	+	+	+	+	+	+	+	+	+
5027	−	+	+	−	+	+	+	+	−	+	+	+	+	+
5030	−	+	+	+	+	+	+	+	−	+	+	+	+	+

viremia on one or more occasions (Table 1). Cat no. 5017 was positive at week 4 and again at weeks 12 and 36. Cat no. 5032 had cell-associated viremia at weeks 12 and 16 and became persistently virus-positive following reinoculation at week 24, while cat no. 5003 had cell-associated viremia only at week 36. The remaining two cats in group 3 never became viremic following either of the two inoculations. By contrast, all five cats in group 4 (FIV-Petaluma) had recoverable virus in their PBMC by 2 to 4 weeks post-inoculation and remained virus-positive for the duration of the study (Table 1).

TABLE 2

Detection of FIV DNA in bone marrow (BM) or lymph nodes (LN) of cats at 12, 36 and 105 weeks following inoculation

Group no.	Infecting virus	Samples positive by PCR: weeks following infection					
		12		36		105	
		BM	LN	BM	LN	BM	LN
1	None	0/5	nt[a]	nt	nt	0/4	nt
2	$FIV\text{-}CrFK_{AZT}$	2/5	nt	nt	0/5	nt	nt
3	FIV-CrFK	1/5	nt	nt	5/5	nt	nt
4	FIV-Petaluma	4/5	nt	nt	nt	5/5	nt

[a]nt, not tested.

TABLE 3

Antibody responses to FIV in experimentally inoculated SPF cats[a]

Cats	Weeks post-inoculation													
	0	2	4	6	8	10	12	16	20	26	28	30	32	36
Group 1 (sham-infected controls)														
All cats	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Group 2 (FIV-CrFK$_{AZT}$)														
All cats	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Group 3 (FIV-CrFK)														
5003	−	−	−	+/−	+/−	−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−
5013	−	−	−	+/−	+/−	+/−	+/−	+/−	−	+	+/−	+	+/−	+
5017	−	−	+/−	+	+	+	+	+	+	+	+	+	+	+
5029	−	−	−	−	−	+/−	−	−	+	−	−	−	−	+
5032	−	−	−	+/−	+/−	+/−	−	+	+/−	+	+	+	+	+
Group 4 (FIV-Petaluma)														
5000	−	−	+	+	+	+	+	+	+	+	+	+	+	+
5010	−	−	+	+	+	+	+	+	+	+	+	+	+	+
5016	−	+/−	+	+	+	+	+	+	+	+	+	+	+	+
5027	−	−	+	+	+	+	+	+	+	+	+	+	+	+
5030	−	+/−	+	+	−	+	+	+	+	+	+	+	−	+

[a] −, Negative at serum dilution 1:10.
+/−, Weakly positive at serum dilution 1:10.
+, Strongly positive at serum dilution 1:10.

Detection of proviral DNA by PCR

FIV DNA was detected in the bone marrow of 0/5 group 1, 2/5 group 2, 1/5 group 3, and 4/5 group 4 cats at 12 weeks following inoculation (Table 2). FIV DNA was no longer detectable in tissues of group 2 cats at the time they were killed at 36 weeks post-inoculation, but was detectable in 5/5 cats from group 3. Tissues from group 1 cats were uniformly negative, while tissues from 5/5 group 4 cats infected with virulent FIV-Petaluma were uniformly positive, even when they were tested 2 years following infection.

Serologic responses

Cats in groups 1 and 2 never developed detectable antibodies to FIV during the 36-week period of the study (Table 3). This was in concordance with virus isolation results, and for cats in group 2 highlighted the negligible infectivity of FIV-CrFK$_{AZT}$. In group 3 all cats eventually seroconverted, but the strongest and most consistent serologic responses were reserved for the two cats from which virus was most often isolated (nos. 5017 and 5032). Seroconversion of all five cats in group 3 was also confirmed by Western blot analysis (data not shown). All five cats in group 4 (FIV-Petaluma) became strongly seropositive by week 4 post-infection and remained positive for the duration of the study (Table 3).

Discussion

In this study we have shown that the FIV infection model could distinguish differences in virulence among three different but related FIV variants. Infectivity, one component of virulence, was determined by assessing replication of the virus in cells of the body at regular intervals following inoculation. This was accomplished in three ways: by isolating the virus from peripheral blood mononuclear cells (PBMC), by detecting FIV proviral DNA in bone marrow or lymph node cells at several time points following inoculation, and by measuring FIV antibody responses. Virus reisolation from the blood is the most direct measure of the levels and duration of virus replication in the host. Detection of proviral DNA by PCR was also a measure of infectivity but not of the ability of the virus to replicate to high levels or for prolonged periods of time. By comparison, the appearance of antibodies was considered as an indirect measure of infectivity. As shown here, the magnitude of the antibody response was proportional to the magnitude and duration of the viremia. The second component of virulence, i.e., the ability of the virus to cause disease signs, was measured by characteristic hematological signs of disease such as neutropenia and changes in absolute and relative numbers of CD4+ and CD8+ T cells in the blood.

The three FIV variants used in this study differed greatly in virulence for cats. The non-cell culture-adapted FIV-Petaluma (group 4) induced a sustained PBMC-associated viremia in all inoculated animals, producing gross and

hematologic abnormalities typical of primary FIV infection (Yamamoto et al., 1988). Adaptation of FIV-Petaluma to growth in CrFK cells led to a decrease in virulence; minor clinical signs of disease were observed but there were no evident hematologic abnormalities. Seroconversion, reflecting the level and intensity of virus isolation, was highly variable and antibody levels were generally low. The AZT-resistant mutant of FIV-CrFK lacked virulence altogether; even though proviral DNA was present in tissues 12 weeks following inoculation, no infectious virions could be recovered from the PBMC and there was no evidence of seroconversion at any time during the study. Moreover, even proviral FIV-CrFK$_{AZT}$ DNA had disappeared by 36 weeks following infection. These findings were not altered by a second inoculation of the virus.

Differences in the above mentioned determinants of virulence were mirrored by the primary disease signs that each virus produced in animals. Signs of the primary stage of FIV infection typically appear from 6 to 8 weeks following inoculation and persist for several weeks (Yamamoto et al., 1988). Gross clinical signs of the primary illness can include fever, generalized lymphadenopathy, and sometimes diarrhea, while hematologic abnormalities include neutropenia, and inversion of the CD4+:CD8+ T-cell ratio. The latter abnormality was associated with an absolute decrease in CD4+ T cells and a normal to increased number of CD8+ T cells; such changes are characteristic (Ackley et al., 1990; Barlough et al., 1991). Hematologic abnormalities usually are proportional in severity to gross clinical signs of illness and are, therefore, the most quantifiable measures of virulence. As expected, the non-cell culture-adapted FIV-Petaluma was fully virulent, inducing significant gross and hematologic alterations. Adaptation of FIV-Petaluma to growth in CrFK cells led to a decrease in virulence, as measured by the inability of the FIV-CrFK virus to induce primary clinical signs of disease, a sustained viremia, and a marked antibody reponse. The AZT-resistant mutant of FIV-CrFK was even less virulent than its parent. It produced no detectable viremia, no clinical signs of disease, and no seroconversion. Nevertheless, the FIV-CrFK$_{AZT}$ mutant was still infectious in vivo based on the ability to detect FIV proviral DNA in tissues of 2/5 inoculated cats at 12 weeks following infection. Unlike infection with FIV-CrFK, however, proviral DNA from FIV-CrFK$_{AZT}$ was not detected in lymph nodes at the termination of the study, 36 weeks following inoculation. Therefore, FIV-CrFK$_{AZT}$ could not sustain itself in the host even as a latent infection.

Several conclusions may be drawn from the results of these experiments. First, it is possible to measure and compare the in vivo virulence of different FIV isolates in experimentally inoculated SPF cats. It is important to note that infected cats need not be monitored for prolonged periods in order to determine an endpoint; the characteristic clinical and hematologic abnormalities evident within the first few months post-inoculation are sufficient and striking enough to compare the biological behavior of one virus strain versus another. Second, cell culture adaptation of FIV-Petaluma may result in

significant decreases in virulence. However, the decrease in virulence may depend upon the cell line in which the wild-type virus is grown and not to the actual process of cell culture propagation. For example, FIV-Petaluma grows very well in PBMC cultures, and virus replicated in such cells appears to retain a major portion of its virulence for cats (Yamamoto et al., 1988, 1991). By contrast, CrFK cell adaptation apparently selects for FIV variants that are much less virulent. Adaptation of field viruses to unnatural host cell types has been widely exploited over the years for attenuation of virus virulence (Norrby, 1989). Third, the present study indicates that AZT selection pressure may select for variants that differ in virulence from parental stock. Although our results suggest that AZT-resistant variants of FIV-Petaluma are less infectious and virulent than wild-type virus, this conclusion must remain tentative at present; the fact that FIV-CrFK$_{AZT}$ was derived from an already attenuated virus strain (FIV-CrFK) makes such an interpretation problematic. Moreover the attenuation observed in vitro following propagation of virulent FIV-Petaluma in CrFK cells and AZT selection may not necessarily represent a general phenomenon applicable to other virulent field isolates of FIV.

Further studies of the type reported here must be designed to control for variables independent of antiviral drug resistance. Ideally the cell culture-adapted virus that is undergoing drug selection pressure should retain all or most of the infectivity and virulence of the wild-type parent. This can best be accomplished by replicating the virus in vitro in PBMC cultures rather than in unnatural cell types. The effect (or lack of effect) of PBMC culture adaptation may be assessed by comparing the infectivity and virulence of the cell culture-adapted strain to its cat-passaged counterpart. Variants selected in vitro for drug resistance should also be compared to their cell culture-adapted parent. To assure that drug-resistant viruses do not revert to wild-type, isolates obtained from the blood should be tested periodically for the drug-resistant phenotype. The infectivity and virulence of a particular drug-resistant mutant can also be assessed in cats undergoing treatment with the antiviral drug in question, i.e., by exerting drug selection pressure in vivo to sustain the resistant phenotype. From our standpoint it may be more relevant, both biologically and philosophically, to create antiviral drug-resistant mutant viruses by in vivo drug selection rather than by in vitro selection in cell cultures.

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